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STUDIES ON THE OXYGEN-SUPPLYING POWER OF THE SOIL TOGETHER WITH QUANTITATIVE OBSERVATIONS ON THE OXYGEN-SUPPLYING POWER REQUISITE FOR SEED GERMINATION*

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(WITH NINE FIGURES)

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I. Introduction

The study of the oxygen-supplying power of the soil is a new one in plant physiology and ecology, although it has long been supposed that the oxygen of the soil influences plant growth. The static aspect of the soil oxygen problem has been attacked by a few investigators working experimentally and by a larger number of writers who have discussed probabilities and expressed opinions without much quantitative data. The whole subject of soil aeration, as thus far developed, is based on the same kind of conception of static conditions as has characterized almost all ecological and physiological study until very recently. Soil moisture and soluble soil salts have been much studied, but generally with the idea that the amount of water or salt actually present in each unit of soil somehow determines the growth of plants rooted therein. In the earlier literature of soil aeration this same idea has generally been followed. While the present paper was in process of preparation there appeared Clements's (**8**) review of this literature, and his book has been carefully studied to determine whether there might be there recorded any discussion wherein attention had been given to the dynamic aspect of soil aeration. But no suggestion of such a method of approach appears in those pages nor is there any mention of this remarkable deficiency.

As has recently begun to be pointed out or implied by a few writers, the environmental conditions that influence plant growth and development do so through the different rates at which the environment can deliver matter or energy to the organism, or take it away. When the possible rate of delivery of any substance, for example, to the plant is higher than the rate at which absorption of that substance needs to proceed for satisfactory growth, then the plant can not suffer from lack of that substance—as far as the environment is directly concerned. Of course the plant may, under the influence of some particular environmental complex, be so constituted or in such a physiological condition as to be unable to absorb some needed substance at an adequate rate for satisfactory growth, even though the present

* Botanical contribution from the Johns Hopkins University, No. 79.

environmental supply be adequate; but in such cases the deficiency is not to be directly related to the environmental supply of the substance in question. It is to be related to the internal characteristics of the plant itself as influenced by the other environmental features. It is clear that an organism can not absorb any substance more rapidly than that substance is supplied from the environment to the absorbing portion of the body periphery. An organism may of course absorb a given substance at any rate that is *lower* than the maximum rate at which the environment can supply that substance. Physiology, ecology, pathology, agronomy, horticulture, and forestry, as well as all other sciences and arts that deal with life as a complex of processes need very much to consider many of their problems from this point of view.

It appears that the only influential environmental conditions thus far studied with reference to supplying power are the evaporating power of the air, that of radiation, and the water-supplying power of the soil. The first two deal with environmental rates of removal of water from the organism; these may be considered as cases where the supplying power is negative. The water-supplying power of the soil has begun to be studied (**9, 15, 17, 19**), but little more than the development of promising methods of thinking and experimentation has yet been achieved in that connection. It seems possible that this dynamic aspect of the soil-moisture problem may now be rapidly developed, and that the conception of environmental supplying powers in general may soon begin to attract more attention. This conception is new, however, with its terminology and all its refinements of thought still to be worked out, while its applications in forestry, agriculture, etc., are as yet undemonstrated excepting in an *a priori* way.

Generally, the logical promise of the supplying-power point of view is so great and the little study that has thus far been devoted to the water supplying power of the soil seems to be so enlightening, that it appeared desirable to make an attempt to devise methods by which the capability of the soil to supply another essential substance might be quantitatively and directly studied. This apparent desirability formed the point of departure for the studies reported in this paper.

Considering the substances that must be supplied from the environment to ordinary plants, and thinking of these with reference to the relative amount of each that is required to bring a plant to maturity, water is surely the one that should be first mentioned. As has been said, fair beginnings have been made toward the dynamic study of the environmental conditions that influence the rates of entry and exit of water from the plant. Next to water, with reference to the amounts needed by green plants, is carbon dioxide. Livingston (**16**) has described a small beginning of the study of

the carbon-dioxide-supplying power of the air but that of the soil has not been considered in the literature. Indeed, the literature appears to offer no suggestion that this soil feature may be important in plant physiology, ecology, etc. Oxygen, uncombined, is third in amount needed, on the list of environmental substances required by ordinary green plants, and second on the list when higher non-green plants are considered. It seems safe to suppose that plants seldom or never suffer in nature from inadequate oxygen-supplying power of the air, but it seems to be clear that differences in the oxygen-supplying power of the soil must be of great importance in determining plant growth in different soils, etc. Consideration of this soil feature lies, of course, in the field of the general problem of soil aeration. It seemed desirable to attack next the oxygen-supplying power of the soil, as a very important environmental characteristic that might be studied dynamically and quantitatively.

Another line of thought was influential in determining the point of attack for the studies here reported. If we consider the substances required by cultivated plants in the order of their possible practical control by plant growers at present, and in the order of their present importance in the selection of sites for plant growing, water is certainly first and soil oxygen is surely second. Growers everywhere can manipulate the water conditions about their plants, or select sites with regard to natural water conditions, more readily than they can similarly deal with any other feature of environmental supply. Watering, irrigation, shading, protection from wind, cultivation, soil drainage, many aspects of pruning, and the selection of plant varieties and of soils to be employed, are all clear illustrations of the practical importance of water relations in plant culture. The soil oxygen relation is also practically considered and manipulated, consciously or unconsciously, especially in cultivation, irrigation and drainage. The carbon-dioxide supply is perhaps involved in some of the usual operations of plant culture, although usually unconsciously so, and carbon dioxide may perhaps be placed as third in the present list. The carbon-dioxide-supplying power of the soil (and perhaps of the air in some cases) is somewhat readily manipulated by cultivation, drainage, irrigation, manuring, etc.

The oxygen-supplying power of the soil was made the subject of these studies because of the considerable quantities of oxygen apparently needed by ordinary plants, because many plants are thought to suffer from an inadequate supply of oxygen from their soil environment, because the soil-oxygen-supplying power may be easily altered by cultural practice, and because this soil feature may readily be consciously and quantitatively considered in selecting plant varieties and soils for cultural operations and in

modifying the operations themselves. What is first needed, apparently, is a method suitable for measuring and comparing the oxygen-supplying powers of different root environments, that is, of different soils. The dynamic water relations of plants have already been fairly well opened for further quantitative study, so that fundamental work in that field seemed to be not so pressingly needed as the general opening of the subject of the oxygen relations of roots. Furthermore, it was thought that the comparative measurement of the oxygen-supplying powers of soils seemed to offer greater practical difficulties than did the corresponding measurements of the carbon-dioxide-supplying powers of soils, and it seemed that suitable methods for the former might involve many features directly applicable to the latter. In point of fact, the method described in the following pages may, with relatively slight alterations, be adapted to the measurement of the carbon-dioxide-supplying powers of soils. Finally, growers of plants and others who deal with plants in scientific research are perhaps generally more nearly ready at present to profit by scientific advances with regard to soil oxygen than they are to make use of advances with regard to soil carbon dioxide.

These studies have to do particularly with methods for measuring and comparing the oxygen-supplying powers of different root environments, primarily soils. In a broader way, the methods here set forth may be valuable for comparative studies of soil-oxygen-supplying powers in other connections (such as that of soil micro-biology, engineering, etc.), and also for the measurement of the oxygen-supplying powers of many other kinds of environment besides soils. Thus, for example, this environmental feature may be studied for different depths in standing or flowing water, as of lakes, rivers and the ocean, for interiors of masses of culture media, etc.

The experimentation was carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, under the personal direction of Professor BURTON E. LIVINGSTON, who has contributed very much to the paper as it now appears.

II. Literature

As has been said, there seems to be almost no literature at all bearing on the question of oxygen-supplying powers of soils. CLEMENTS'S (8) very thorough-going summary of the literature of soil aeration presents an apparently nearly complete review of the subject up to about 1920, but a thorough study of that monograph has not brought to light any references to oxygen-supplying power. Many writers have dealt with the different oxygen *contents* of various soils and at various depths in the soil, and the general idea of the environmental supplying power has doubtless been in

many minds. But the only statement bearing directly on this aspect of the soil oxygen question thus far encountered in the extensive literature of soil oxygen and the oxygen requirement of plants is one by CANNON (**3**), who says: "It is the rate of supply and not the partial pressure of the gas (in the soil air) that is important." It is of course true that the partial pressure of oxygen in a root environment may in many cases be considered as directly proportional to the environmental supplying power for that substance, but this can be regarded as true only for special cases. A soil containing but little oxygen per unit of volume might nevertheless be capable of delivering oxygen to the absorbing surfaces of roots at a considerable maintained rate, while a soil containing relatively much oxygen might soon become depleted near the absorbing surfaces and its maintained rate of delivery might consequently be low.

It will not be necessary or desirable to attempt here to summarize the literature of soil aeration further, since supplying powers have not hitherto been considered. A number of papers on the static aspect of this subject have appeared since the publication of CLEMENTS'S monograph, one of the most satisfactory of which is that of ROMELL (**18**). That paper is worth careful study, but it does not bear directly on the dynamic problem with which the present paper deals. A few papers that bear on the discussions of the present results will be mentioned in the following pages. A preliminary statement of the aims of these studies and of the methods used has been published by HUTCHINS and LIVINGSTON (**11**).

III. Method and apparatus

1. General considerations

The determination of the capacity of a soil to supply elementary oxygen would require two essential units in the apparatus, an absorber and an indicator. The absorber should present to the soil a known area of absorbing surface and should be able to absorb oxygen as rapidly as the latter arrives at its surface, and the indicator should furnish means for determining the mean rate at which absorption has been occurring during a given test period. The rate at which the absorber receives oxygen would be taken as a measure of the power of the surroundings to supply that element.

The absorber here used is made by altering in a suitable manner a porous porcelain cone of the type described by LIVINGSTON (**14**) for use in the auto-irrigation of potted plants. The cone is embedded in the soil at any desired depth, oxygen entering by diffusion through water of imbibition held in the porous-porcelain wall, which is constantly kept wet enough to prevent any flow of gas through it. The cavity of the cone is kept practically free of oxygen by means of a stream of oxygen-free gas that carries the absorbed

oxygen from the absorber to the indicator. The gas flow is maintained constant by pressure and orifice controls, and is so regulated that it moves rapidly enough to sweep out the absorbed oxygen as it arrives in the absorber, but not too rapidly to allow the indicator to account for all of the oxygen that reaches it.

The oxygen brought to the indicator by the gas stream is absorbed by a standard quantity of a standard aqueous solution of pyrogallol and potassium hydroxide, and the rate at which absorption takes place here is taken as a measure of the rate of entrance of oxygen into the gas stream from the walls of the absorber. The rate of oxygen absorption by the indicator solution is determined colorimetrically, advantage being taken of the fact that this solution slowly becomes darker colored as its oxygen content is gradually increased. A test is begun with the color of the indicator solution exactly matching a permanent color standard and the test consists in determining the time needed for the solution color to become the same as that of a second standard darker than the first. A definite quantity of oxygen, which may be called the *colorimetric oxygen unit* for this indicator, is required to bring about this color change in a charge of indicator solution, and each test shows the number of minutes required for the absorption of one of these units of oxygen from the soil by the absorber. The several rates of absorption indicated for a series of tests are consequently considered as proportional to the reciprocals of the corresponding lengths of the test periods. Thus, if the specified color change in the indicator solution should require 15 minutes for one soil test and 29 minutes for another, the oxygen-supplying powers of the two soils would be taken as 1/15 and 1/29, respectively; that is, the second soil could supply oxygen only 15/29 as rapidly as the first.

The several parts of the apparatus will be described in detail and discussed, and then a section will be devoted to additional notes on the operation of the apparatus.

2. The absorber

The absorber is a hollow cone of porous porcelain, and oxygen is absorbed by diffusion through a known area of its wet curved surface. It is prepared from a Livingston (**14**) porous porcelain auto-irrigator cone by grinding the surface until the thickness of the curved wall is about 0.5 mm. The grinding is done roughly at first by means of a coarse emery cloth held against the surface of the cone as the latter is rapidly revolved on a motor-driven spindle. For later stages in the grinding the cone is stationary and a motor-driven flexible shaft and sandpaper disks such as those used in dentistry are employed. As a guide to securing uniform thickness of wall,

the interior of the cone is illuminated by a small electric lamp during the finishing process. This operation is carried on in a darkened room and proceeds until the light transmitted through the porcelain wall appears uniform over the whole curved surface of the cone and is about equal in intensity to light from a similar source transmitted through ten sheets of Whatman's filter paper no. 30. The plane base of the cone is ground until it transmits light faintly, it being desirable to have the cone walls hold as small a volume of water as is practicable, in order to prevent too great a lag in the tests. The neck and the flat base, and a little of the conical surface above and below are next coated with sealing wax ("Dennison's Fine Red") to confine oxygen absorption to the thin, curved surface. The construction of the absorber now used, with its stopper and tubing connections,

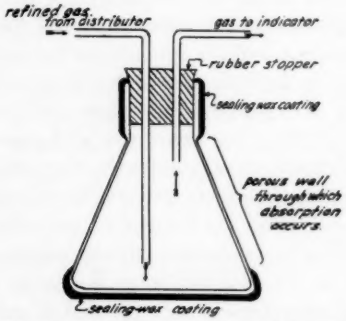

FIG. 1. Diagram showing vertical section of absorber.

is shown by diagram in fig. 1. Each absorber is given a number, and the measured area of its curved absorbing surface is recorded.

The thin, porous wall of the absorber needs to be impregnated with a substance such that the wall remains permeable to the movement of gas by diffusion but is impermeable to mass movement or streaming. Refined paraffin oil ("Nujol") was found to be well adapted for this purpose with absorbers exposed to dry environments (**11**, p. 135), but most soils that are moist enough to make a study of their oxygen-supplying powers of interest in physiological and ecological connections, also supply enough moisture to keep the wall of the absorber sufficiently impregnated with water (soil solution). This moisture is held in the porous porcelain by a strong imbibitional force, pressures of an atmosphere or more being required to cause gas streaming through the wall when the latter is wet. It is of course possible that the imbibed moisture may become depleted if the surrounding soil is very dry, but one of the mercury valves in the apparatus would always show clearly if the gas were to begin to stream through the wall of the

absorber. Gaseous movement between the environment and the cavity of the absorber is to take place only by diffusion through the water held in the absorber wall.

When ready for operation, the neck of the absorber is closed by a rubber stopper bearing two tubes. One tube conducts the gas stream, of oxygen-free nitrogen, into the bottom of the absorber; and the other tube conducts the gas stream, which now generally bears some oxygen, out of the top of the absorber to the indicator, where the time-period for the delivery of an indicator unit of oxygen is determined. The oxygen-supplying power of the environment being tested is calculated from the length of this time period and the area of the absorbing surface. It is expressed in terms of the quantity of oxygen delivered in a unit of time per unit of area; as, *e.g.*, milligrams of oxygen supplied per hour through a square meter of absorbing surface exposed to the soil.

3. The gas stream

(a) Source of gas and initial control.—In the preliminary part of these studies (**11**) the source of the gas stream was the laboratory supply of illuminating gas, the free oxygen in the latter having been removed by alkaline-pyrogallol solution in scrubber bottles and pressure gradient for movement having been supplied by a bottle aspirator. For the later work commercial nitrogen (furnished under pressure in tanks) has been found to be superior and more convenient. The parts through which the gas passes before reaching the absorber may be considered in the order in which the gas reaches them.

Nitrogen from the supply tank passes through the partially open throttle (fig. 2, valve 1) into the chamber of the throttle control valve (fig. 2, valve 2). The gauge of this valve indicates the pressure of the gas as it leaves

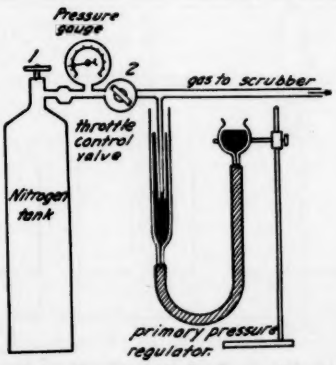

Fig. 2. Diagram showing primary control of gas-stream from nitrogen tank.

the valve. This pressure and the rate of flow are regulated by adjusting the valve and a very uniform slow rate is readily secured. The throttle itself should not be opened wider than is necessary; the pressure indicated by the gauge should be between 200 and 500 pounds. Adjustment is made by means of the throttle and the control valve from time to time as the gas pressure in the supply tank gradually decreases.

A lateral branch of the tube leading from the control valve is connected to a primary mercury pressure-regulator, shown by the diagram of figure 2. This branch extends downward into a vertical glass cylinder graduated for height (an ordinary burette is convenient). The lower, open end of the branch is closed by mercury in the cylinder and the mercury level is regulated by raising or lowering a properly supported mercury reservoir (thistle tube) connected by rubber tubing with the lower end of the cylinder. The height of the mercury column in this regulator is always such that (1) gas pressures in succeeding parts of the apparatus will never be great enough to expel stoppers or cause other damage and (2) sufficient pressure is maintained to cause continual gas-escape from the secondary pressure-regulator, which will be described later. The main tube from the throttle-control valve leads to the scrubber.

(b) THE SCRUBBER.—Commercial nitrogen contains some oxygen and the latter must of course be removed before the gas enters the absorber in the soil. This is accomplished by means of aqueous alkaline pyrogallol solution in the scrubber and in the refiner. The scrubber is composed of several like units connected in series, each unit consisting of a glass bottle of about four liters capacity, containing about a liter of scrubber solution

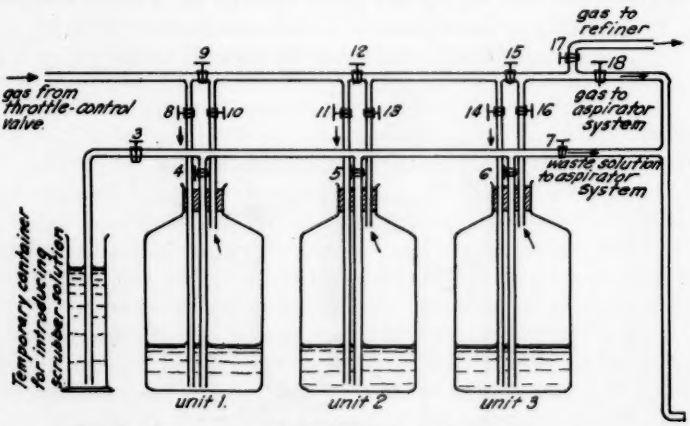

FIG. 3. Diagram showing arrangement of scrubber parts.

and closed by a rubber stopper bearing three tubes, one for introducing or removing liquid, another for entrance of gas from the throttle-control valve, and the third for exit of gas. The scrubber arrangement is shown diagramatically in fig. 3. The gas inlet tube reaches nearly to the bottom of the bottle and the outlet tube extends only a short distance below the stopper. The outlet tube is connected to the next scrubber unit (in the case of the last unit, to the refiner). The number of scrubber units needed depends upon the amount of oxygen in the original gas supply and on the rate of flow. For commercial nitrogen used in these studies and for the continuous operation of eight absorbers four scrubber units have been found to be adequate.

Two forces operate to move the liquids and the gas, (1) the pressure of the gas as it leaves the throttle-control valve and (2) suction produced as needed by an aspirator (Chapman filter pump) operated by the laboratory water supply and connected by tubing to the various parts of the apparatus where aspiration is needed. To remove and replace the solution in a scrubber unit it is necessary that the movement of gas through that unit be temporarily stopped. The scrubber is therefore so arranged that, by closing the gas inlet and outlet cocks of any unit and opening a by-pass, the unit in question may be disconnected from the system without interrupting the gas stream as a whole. The operation of removing and introducing scrubber solution is accomplished without admitting any air to the unit, and the latter is then again connected with the system.

The scrubber solution is prepared within each unit by mixing an aqueous solution of pyrogallol and one of sodium hydroxide. About 250 cc. of a 15-molecular solution of the hydroxide is passed into the unit and this is followed by about 750 cc. of a 4-molecular solution of pyrogallol. [See ANDERSON (**1**)]. For the present purpose the solution described in the text has been found to be more satisfactory than the one recommended by ANDERSON. The hydroxide solution is prepared by dissolving in distilled water the entire contents of an original 1-lb. package of sodium hydroxide to make 600 cc. of solution. For the pyrogallol solution 500 grams of the crystals is dissolved in distilled water to form a liter of solution.

To introduce one of these solutions, or water for cleansing, into a scrubber unit, the liquid to be introduced is first placed in a scrubber-supply container (fig. 3) graduated so that a known amount can be drawn into the liquid conduit, which is extended for this purpose. Starting with the units empty and all cocks of the scrubber-system closed, aspiration is applied to all the units by opening the cocks in the gas outlet tubes and the by-passes. After air has been removed as far as possible, and while the aspirator is still functioning, the first cock in the liquid conduit (cock 3) is opened, as is

also the cock in the branch of that conduit (cock 4) leading into the first scrubber unit. The solution from the container now begins to flow into the first unit and the flow is stopped by closing cock 4 when the desired amount (250 cc.) has been transferred. Similarly, the cocks in the branches of the liquid conduit leading to succeeding units are successively opened to allow introduction of the hydroxide solution and are closed after the proper amount has entered. The excess of hydroxide solution left in the graduated container is now replaced by pyrogallol solution, the container being first thoroughly rinsed, and the required amount (750 cc.) of the latter is passed into each unit by the method described for the introduction of hydroxide solution. The conduit for liquids and its branches are now washed with distilled water. Thorough mixing of the two solutions may be hastened by gently agitating each scrubber bottle. All open cocks of the scrubber system are to be closed. Each unit now contains the proper amount of fresh solution but, because of the continued action of the aspirator during the introduction of the solutions, their internal gas pressure is likely to be far below that of the external atmosphere. To relieve the partial vacuum, nitrogen is next introduced into the units from the throttle-control valve in the following manner: The throttle-control valve is slowly opened until gas escapes freely but not violently from the primary pressure-regulator. The first cock of the gas conduit (cock 2) is opened and then the cock of the gas inlet to the first scrubber unit (cock 8) is opened *cautiously*, care being taken that mercury or air is not drawn in from the regulator. When the gas pressure in the first unit has become slightly greater than that balanced by the mercury column in the regulator, as is indicated by gas escaping from the latter, the cock controlling the gas outlet from that unit is opened. Then the cock controlling the gas inlet for the second unit is *cautiously* opened and this unit is supplied with nitrogen. The same procedure is followed for this and succeeding units as has been described for the first unit. After all units have been cared for the cock in the gas conduit leading from the scrubber (cock 17) is opened, allowing the gas to pass on to the refiner.

Since the gas from the supply tank passes through the scrubber units in series, the power of the scrubber solution to absorb oxygen decreases more rapidly in the first unit than in succeeding ones, and the solution in the first may have lost most of its absorbing power while that in the other units still remains very potent. To judge of the condition of the scrubber solution in any unit, the gas connections of that unit are closed and a small amount of the solution is passed, by suction from the aspirator, up into the conduit for liquids, which is a glass tube of about 5 mm. bore, and is viewed in ordinary light. If the sample appears opaque the oxygen-absorbing

power of the solution in that unit may be considered as low and a renewal of solution is desirable. In each unit the vertical branch of the conduit for liquids is usually filled with water, as is also the conduit itself, and to bring the sample into the conduit for observation it is necessary to allow the contents of the vertical branch to flow into the conduit first, letting it be followed by the sample. If this test indicates that a change of solution is not yet necessary the solution should of course be removed from the conduit and the latter filled with water drawn from the scrubber-supply container. Finally, the gas connections are opened, placing the tested unit again in operation.

If the sample drawn up into the liquid conduit appears opaque, the unit in question should be emptied, the waste solution being discarded through the aspirator system. After the solution has just been removed the gas pressure in the unit is low, as it was before the unit was originally charged. Aspiration is now cut off (cock 7) and the unit is recharged by transferring into it the solution from the next succeeding unit, the gas connections of which must first be closed, assuming that that solution is still potent. The unit thus emptied is first partially exhausted by means of the aspirator system operating on the liquid conduit, aspiration is again cut off (cock 7) and the unit is recharged as before from the next succeeding unit. Finally the last unit is recharged with new solution, introduced from the supply container in the manner described for originally charging the scrubber units. By this method of procedure the most potent oxygen-absorbing solutions are always in those units nearest to the refiner, to which the gas passes after leaving the scrubber. After all emptied units have been recharged the gas connections are once more opened and the entire scrubber series goes again into operation.

To illustrate a method of transferring scrubber solutions, let it be assumed that the solution in the first unit is to be discarded, that the solutions in the second and third units are to be transferred to the first and second units, respectively, and that fresh solution is to be prepared in the third unit. First, all open cocks of the scrubber system are to be closed. Aspiration is applied to the conduit for liquids and then the cocks that control the liquid conduit between the first unit and the aspirator system are opened (cocks 7 and 4). Solution passes from the first unit into the conduit for liquids and is discarded through the aspirator system. Aspiration is continued until all of the liquid and as much gas as possible has been removed from the first unit. The exhaust is now cut off from the conduit for liquids by closing cock 7. To pass the solution from the second unit into the first, the cock controlling the branch of the conduit for liquids leading into the second unit (cock 5) is opened, permitting the gas pressure above the solu-

tion in that unit to force the solution over into the first unit. The gas pressures in the two units are allowed to become adjusted, then the cock controlling the branch of the conduit for liquids leading into the first unit (cock 4) is closed and the cock controlling the branch of the conduit for liquids leading into the third unit (cock 6) is opened, allowing the solution to pass from the third into the second unit. Again gas is allowed to follow the liquid until the pressures in the two units are adjusted. Fresh scrubber solution is now introduced into the third unit, in the manner already described. Then the conduit for liquids and its branches are rinsed with water and the gas from the throttle-control valve is allowed to adjust the pressure in all of the units. The cock in the gas conduit leading from the scrubber (cock 17) is opened, permitting the gas to pass on to the refiner. Should it happen that only a part of the solution from one unit has passed into the preceding unit of the scrubber series when the gas pressures in the two units become equalized and the solution ceases to flow, as is particularly apt to be the case in transferring solution from the last unit of a scrubber, the gas in the unit receiving the liquid is rarefied by opening the necessary cocks and applying the exhaust through the gas conduit, the by-passes and the gas outlet tube of the partially charged unit, until all of the solution has been transferred.

Ordinarily one charge of solution in the units should suffice to remove the oxygen from the gas in one nitrogen tank of the size and compression used in this work, and it should not usually be necessary to test the scrubber solution excepting when a new cylinder is introduced.

Cylinders of nitrogen received from the factory are each calculated to contain a quantity of nitrogen such that, if allowed to expand under atmospheric pressure, the gas would occupy a volume of 100 cu. ft. If the requisite rate of gas flow is taken as 10 cc. per minute, which is greater than the actual requirement in the studies here reported, a single cylinder of gas should last about 185 days, with eight absorbers in continuous operation. Commercial nitrogen may contain as much as 1 per cent. of oxygen, and it may consequently be roughly estimated that the scrubber should be capable of removing oxygen from the gas stream at a continuous rate of 0.01 cc. (atmospheric pressure) per minute. With several scrubber units in series a considerable reserve of scrubbed gas is generally available and it is consequently possible to pass rapidly a liter or more of oxygen-free gas into other parts of the apparatus whenever that may be necessary.

(c) SECONDARY PRESSURE REGULATOR.—The secondary pressure-regulator is inserted in the main gas conduit between the scrubber and the refiner. Its arrangement is shown by the diagram of fig. 4. It is similar to the primary pressure-regulator. An open branch of the conduit is ex-

tended into a vessel of mercury, the end of the tube reaching to a depth so that the mercury pressure will correspond to a pressure on the gas at this point slightly greater than that necessary to maintain gas flow. Gas is always slowly escaping from this regulator, more rapidly when for any reason the pressure of the incoming gas becomes a little too high. Only a small amount of gas is thus wasted, however, and the regulator makes it certain that the gas pressure from this point forward in the gas stream can never be higher than that for which the regulator is adjusted.

Coming from the scrubber, the scrubbed gas passes the secondary pressure-regulator and proceeds to the refiner, which removes any traces of oxygen that may have escaped the action of the scrubber.

(d) THE REFINER.—The refiner consists simply of a series of three Pettenkoffer tubes of aqueous alkaline pyrogallol solution so placed that the gas bubbles traverse about two meters of liquid in passing through the three tubes. By means of gas supply from the scrubber, liquid supply from the mixer (to be described below) and a waste conduit of the aspirator system, the solution, and water for cleansing, may be passed into or discarded from the refiner tubes individually with only brief interruption of the gas stream. The refiner and its connections are shown by the diagram

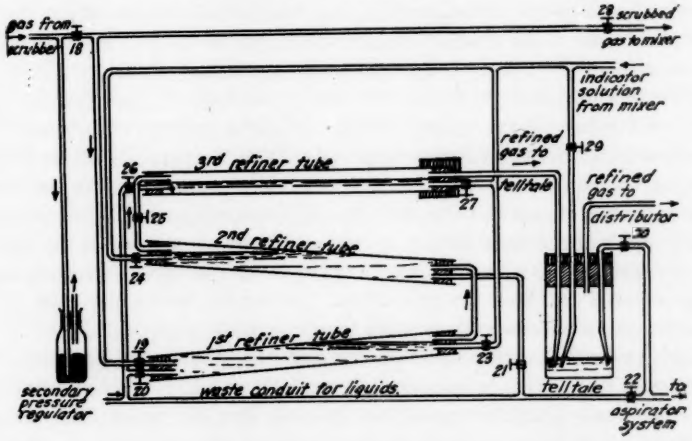

FIG. 4. Diagram showing arrangements of secondary pressure-regulator, refiner and telltale.

of fig. 4. As in the case of the scrubber, the cocks for controlling the liquid conduits consist of Hoffman tubing clamps on heavy-walled rubber tubing, but glass stop-cocks are used to control the gas conduits of the refiner sys-

tem and of all succeeding parts of the gas system. Furthermore, each connection between parts of the gas conduit from the refiner forward is adequately protected against gas diffusion by a mercury seal or by a glass sleeve poured full of sealing wax or paraffin.

The alkaline pyrogallol solution for the refiner is somewhat different from that used in the scrubber, being prepared with potassium hydroxide instead of sodium hydroxide and being more dilute than the scrubber solution, with more sensitive color response when oxygen is absorbed. It is prepared in the mixer in the same general way as is the indicator solution itself. A refiner solution composed of one part of the stock potassium hydroxide solution and two parts of the stock pyrogallol solution is satisfactory. The preparation and use of these stock solutions is dealt with later, where the indicator solution is described.

The refiner is put into operation, starting with all its cocks closed and its tubes containing only air, in the following manner. The cocks are opened that control (1) the gas conduit between the second and third refiner tubes (cock 25), and (2) the waste conduit leading from the telltale bottle (cock 30), which is the next unit in the series (see sec. e). Next the cocks nos. 23, 24, and 27 controlling the liquid inlets are opened and allowed to remain open long enough to permit solution from the mixer to force the air out of the conduits for liquids. The cock (no. 19) controlling the gas inlet to the refiner is now partially opened, allowing gas from the scrubber to enter, thus sweeping the air in the refiner tubes out through the waste conduit of the telltale bottle. When a volume of nitrogen has been passed into the tubes somewhat greater than the capacity of the latter, under atmospheric pressure, the cock (no. 19) controlling the gas inlet of the refiner is closed and the cock (no. 23) controlling the liquid conduit leading to the first refiner tube is opened and remains open until the refiner tube is nearly filled with solution. Of course the solution meniscus must not be allowed to reach the gas outlet. As liquid enters the tube it replaces the gas, which escapes through the gas conduit and the telltale bottle. Similarly, solution is introduced successively into the second and third refiner tubes, using the proper cocks (nos. 24 and 27). When all three refiner tubes are ready for operation, the cock (no. 19) controlling the gas inlet to the first refiner tube is opened, it being first ascertained that the gas pressure in the conduit is correct, as shown by the bubbling of gas out of the secondary pressure regulator. Gas bubbles move through the refiner tubes and the refined gas escapes through the telltale bottle. If the refiner solution is much discolored it is allowed to remain in the tubes about 20 minutes to permit absorption of residual oxygen left in the tubes at the time of filling, after which it is discarded and the tubes are refilled with fresh solution from the mixer.

The method of discarding and introducing solution after the refiner is ready for operation (or has been operating) is as follows: All open cocks (nos. 19 to 27, inclusive) of the refiner are to be closed. The two cocks (nos. 20 and 19, respectively) that control the waste conduit from the first tube and the gas inlet to this tube are opened. Gas enters the first tube from the scrubber and forces the liquid out through the waste conduit and aspirator. The cock (no. 19) controlling the gas inlet to this tube is then closed and the cock (no. 23) controlling the liquid inlet is opened to permit the desired amount of solution to enter, after which the latter cock is again closed. To remove the solution in the second refiner tube, the cock (no. 21) controlling the waste conduit from this tube is opened, as is also the cock (no. 19) controlling the gas inlet to the whole refiner. Gas now bubbles through the first refiner tube, enters the second and there displaces the solution as the latter passes into the waste conduit. To introduce fresh solution into this tube the two cocks (nos. 25 and 24, respectively) that control its gas outlet and solution inlet are opened, to allow the desired amount of solution to enter from the mixer, after which the liquid inlet cock (no. 24) is again closed. Gas from the second refiner tube now passes into the third. To discard the solution from that tube, the cock (no. 26) controlling its waste conduit is opened. Gas now bubbles through the first and second tubes into the third, forcing the liquid out through the waste conduit. The cock (no. 26) controlling this conduit is now closed, as is also the cock (no. 25) controlling the gas inlet to the third refiner tube, and the cock (no. 27) controlling the liquid inlet is opened and remains so until the desired amount of solution has entered the tube, forcing the gas out through the telltale bottle. The entire refiner is now put into operation by cautiously opening the cock (no. 25) that controls the gas conduit between the second and third refiner tubes. Gas from the scrubber should now bubble through the three refiner tubes in series and then pass on to the telltale bottle.

(e) THE TELLTALE.—The telltale (fig. 4) furnishes a continuous test for the presence of oxygen in the gas after the latter has passed through the refiner and is about to be distributed to the absorbers. The telltale is a rubber-stoppered bottle with a sealing-wax seal and with four tubes leading into it as follows: (1) for the entrance of gas from the refiner; (2) the exit of gas to the distributor; (3) for the introduction of solution from the mixer; and (4) discharge of solution through the aspirator system. Gas from the refiner enters the telltale bottle at a point near the bottom of the latter, bubbles through a layer of solution about a centimeter deep and leaves near the top of the bottle. The solution used here is the same as that used in the indicator, to be described later.

Throughout all tests the solution in the telltale bottle should remain without appreciable change in color. Any discoloration of the solution indicates that some oxygen is passing the refiner and if this ever occurs, the refiner, the scrubber, or the gas connections require attention. Whenever this solution has darkened (it may acquire a light orange-brown color after several days) it should be renewed with fresh indicator solution from the mixer, and it is generally well to make this renewal at least every five days.

To place the telltale bottle in operation, beginning when it contains only ordinary air, refined nitrogen is first passed through it for about 15 minutes, the air being thus swept out and discharged through the aspirator system. Indicator solution is then introduced by opening the cock (no. 29) in the solution inlet, which is afterwards again closed.

When the telltale bottle has been in operation and its solution is to be removed, the two cocks (nos. 29 and 30, respectively) in the solution inlet and solution outlet are opened. Fresh solution from the mixer flows into the bottle while solution is at the same time flowing out, and both of these cocks remain open until the solution in the bottle shows the same color as the solution entering from the mixer, then both these cocks are again closed. During the process of renewal, the cocks are to be adjusted so as to maintain the solution level in the bottle approximately constant. The gas flow through the telltale continues uninterrupted while the solution is being renewed. The refined nitrogen flows through the telltale to the distributor, on its way to the absorbers.

(f) THE DISTRIBUTOR.—The distributor consists of a number of units, one for each branch that leads from the main gas supply, from the telltale, to an absorber. Each unit consists of two mercury-valve devices, one allowing the branch to be opened or closed by a simple operation, the other eliminating the possibility of any backward movement of gas from the absorber. The arrangements of the distributor are shown diagrammatically in fig. 5. The device for starting and stopping the gas flow consists of a vertical hollow cylinder of "Pyrex" glass, measuring 12 cm. in length and

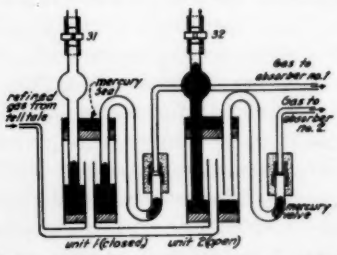

FIG. 5. Diagram showing arrangement of distributor parts.

3 cm. in diameter, closed at each end by a rubber stopper, the upper stopper being inserted far enough to allow for a mercury seal above. A branch tube from the main gas supply, leading from the telltale, perforates the lower stopper and extends upward into the cylinder nearly to the upper stopper. The upper stopper bears an S-shaped glass tube, the inside diameter of which is at least 7 mm. One end of this tube reaches down into the cylinder nearly to the lower stopper, while the other end is vertical for a distance of several centimeters and is finally connected directly with the absorber. The upper stopper also bears a straight vertical glass tube which extends downward into the cylinder to within about 3 mm. of the lower stopper. This tube is expanded into a small bulb above the mercury seal at the upper end of the cylinder, and its upper end is closed by a piece of rubber tubing bearing a Hoffman clamp. In the cylinder is a layer of mercury with its upper surface four or five mm. above the end of the S-tube, which it closes.

The device for preventing reflux of gas from the absorber consists of a plug of cotton supporting a mercury plug about 5 mm. high placed in the final vertical arm of the S-tube just below its union with the tube that leads to the absorber.

To open the unit and allow gas to flow from the telltale to the absorber, suction is applied to remove mercury from the cylinder by transfer to the vertical tube and its bulb, so that the mercury surface in the cylinder falls below the end of the S-tube, thus opening the latter and allowing gas to flow from the gas conduit through the cylinder chamber and the S-tube, and so on to the absorber. The clamp above the bulb is closed and the mercury remains in this position. The mercury resting on the cotton plug at the outer end of the S-tube is agitated as the gas bubbles pass around it and serves as a valuable indicator to show whether the gas is flowing properly. To close the unit, the clamp is opened and the elevated mercury returns to the cylinder, closing the S-tube.

Refined gas from the telltale passes through each open unit of the distributor to the corresponding absorber, from which it moves, through an orifice control, to the selector. By means of the latter it is directed either to the outside, being thus allowed to escape into the atmosphere, or to the indicator where the colorimetric tests are carried out.

(g) THE PRIMARY ORIFICE CONTROLS.—Into the gas line leading from each absorber is introduced an orifice control, a device for controlling the rate of gas movement. This consists of a glass tube about 4 cm. long and 3 mm. bore, containing a plug of firmly packed, dry kaolin, guarded on each side by a plug of cotton. The device is shown in the diagram of fig. 6. These primary controls are inserted in their respective gas line by ordinary rubber-tubing connections thoroughly bound-on, and the whole con-

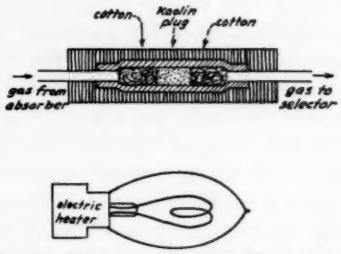

FIG. 6. Diagram showing orifice control.

nection is in each case surrounded by a glass sleeve poured full of sealing-wax. Before each control is inserted its kaolin plug is tested and adjusted as to length so that, with the gas pressure dealt with, the desired rate of gas movement is secured. The kaolin plug should be packed very firmly in the tube and adjustment is made by adding or removing kaolin so as to lengthen or shorten the plug. With the apparatus used in these studies each plug was adjusted so as to allow about 0.8 cc. of gas to pass through it per minute.

Lest the kaolin might sometimes be cool enough to collect liquid water, by condensation, from the passing gas, it is well to maintain the temperature of all the orifice control tubes somewhat above the maximum temperature expected in the absorbers and in the gas tubes leading from them to their primary orifice controls. This is conveniently done by allowing the control tubes to lie side by side across the top of a small wooden chamber in which is kept in constant operation a small electric lamp, the tubes being protected from too intense heat by means of paper lying just beneath them and above the lamp.

Besides the primary orifice controls for the gas streams from the several absorbers, there is also included in this arrangement a similar orifice control for a stream of purified nitrogen, led from the refiner directly to the selector.

(h) THE SELECTOR.—The gas from any absorber, or from the refiner, after passing through the corresponding primary orifice control, may be directed into the indicator cell by means of the selector or it may be released into the atmosphere. The selector is an arrangement by which the indicator may be effectually connected or disconnected at will with any one of the nine tubes that lead gas to this point in the apparatus. Several absorbers are generally in operation at the same time, the gas passing through each one being conducted by its own tube to the selector. Ordinarily the gas streams from all absorbers empty continuously into the air at the selector. Any one stream may be selected here and directed through

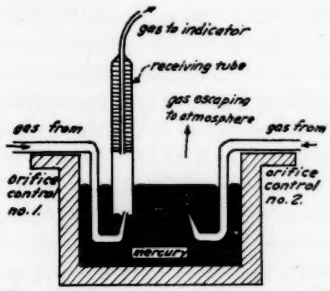

FIG. 7. Diagram showing arrangement of selector parts.

the indicator while the rate of oxygen delivery from the corresponding absorber is being determined.

The arrangement of the selector is shown in the diagram of fig. 7. The device consists of (1) a cylindrical glass vessel about 8 cm. in diameter and 4 cm. deep, containing mercury to a depth of 2 cm., (2) a series of glass escape-tubes, one for each primary orifice control, (including the control for purified nitrogen from the refiner as well as those for gas from the several absorbers) each of which penetrates into the mercury near the edge of the vessel and terminates with an upwardly directed orifice 1 mm. in diameter about 5 mm. below the free mercury surface, (3) a vertical glass receiving-tube about 6 mm. in diameter with its lower end dipping into the mercury, this tube being continued by means of a lead tube of about 1 mm. bore that leads directly to the gas entrance of the indicator cell. The lead tube is about 30 cm. long and its flexibility allows the glass receiving-tube to be moved about and placed over one or another of the gas orifices without lifting its lower end from the mercury. A plug of dry cotton guards the opening from the receiving-tube into the lead tube. Ordinarily the receiving-tube rests over the orifice for purified nitrogen and a stream of this gas flows continuously through the indicator. When the rate of oxygen supply from a given absorber is to be determined, the receiving-tube is lifted slightly, moved laterally through the mercury and lowered over the orifice for the absorber that is to be tested. When a determination is complete the receiving-tube is shifted to the orifice for another absorber or is returned to the orifice for purified nitrogen.

To guarantee an efficient mercury seal about the vertical glass receiving-tube of the selector, care must be taken that *clean* mercury is always in contact with *clean* glass. The mercury-air surface of the selector should not be allowed to become dusty or to accumulate a film that may adhere to the glass wall of the receiving-tube as it is lifted and moved through the mercury; for even with a mercury seal a centimeter in height, if the con-

tacts are not clean, oxygen may diffuse along the glass wall of the receiving-tube and enter the gas stream at a rate sufficiently rapid to give a test-time of a few hours. Whenever the blank nitrogen-check test produces any color change in the indicator solution and the telltale indicates during the same period that the gas stream as it enters the distributor is free from oxygen, attention should be given to the selector before suspecting leaks elsewhere. A guard of cardboard is usually kept over the well of the selector and this serves to protect the mercury surface from dust and to prevent droplets of mercury from being thrown from the selector by the bursting gas bubbles from absorbers that are not being tested.

Actual entrance of oxygen into the gas stream through a leak in the mercury seal of the selector is not likely to occur except after long periods of continuous operation and this difficulty should be entirely avoided by frequently cleaning the glass receiving-tube and the mercury-air surface, as with a piece of moist chamois skin. Occasionally it may be necessary to remove the mercury from the container and to thoroughly clean the mercury, the container, the glass inlet tubes and the glass receiving-tube.

The ordinary laboratory method of cleaning mercury by passing it through a pin-hole in paper held in a funnel has been found satisfactory in these studies, providing the mercury does not contain considerable amounts of amalgam; washing by dilute nitric acid is rarely necessary.

4. The indicator

(a) Indicator cell, check cell and gas-escape cell.—Determination of the rate of delivery of oxygen in the gas stream from an absorber is made in the indicator cell. The indicator system of which this cell is a

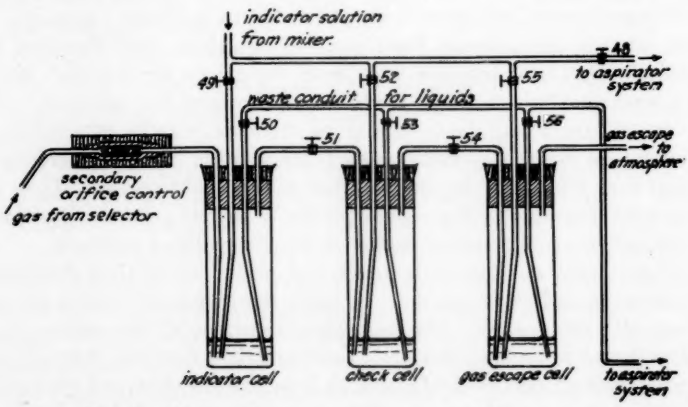

Fig. 8. Diagram showing arrangement of indicator parts.

part is shown by diagram in fig. 8. The system consists of a secondary orifice control similar to the ones already described, but with somewhat less kaolin resistance, and three vial cells, namely, the indicator cell, the check cell, and the gas-escape cell. The three cells are so constructed and connected that a measured quantity of indicator solution may be introduced from the mixer into any cell and subsequently discarded through the waste conduit for liquid. Gas from the selector passes through the secondary orifice control and then through the three cells in the order named, finally escaping into the atmosphere from the gas-escape cell. The three cells are alike in construction. Each consists of a flat-bottomed, cylindrical glass vial about 2.2 cm. in diameter and 6.5 cm. high, closed at the top by a hermetically sealed rubber stopper traversed by four tubes. One tube is for gas entrance and extends to the bottom of the cell, another is for gas exit and terminates just below the stopper. One of the remaining tubes is for the introduction of indicator solution into the cell, and the other is for the removal of solution, both of these extending to the bottom of the cell. The three tubes that reach to the bottom are somewhat bent so that their orifices lie near the lateral wall, thus leaving the mass of solution as it stands in the cell free from obstruction excepting near the right and left sides. The gas outlet from the first cell is connected to the gas inlet to the second and that from the second is connected to the inlet to the third. Each of these connections has a stopcock (nos. 51 and 54, respectively). The vial of the indicator cell has an India-inked file mark on the front and another on the back side, so placed that the center of the meniscus of 1.4 cc. of solution, as it stands in the cell, lies in the plane of the two marks. These calibration marks make it possible to operate the cell always with the same volume of solution. The measurement of the amount of solution placed in the cell might be more precise, but the precision obtained in the way here indicated has been found to be sufficiently great for the work in hand. The check cell and the gas-escape cell require no calibration.

Air is first swept from the three cells by passing a stream of purified nitrogen through them from the selector, cocks 51 and 54 being open. When the originally contained air has been displaced by nitrogen, indicator solution is introduced from the mixer. To introduce a charge of solution into the indicator cell, cocks 37 and 49 are opened, cocks 48, 52, and 55 being closed. The gas stream is allowed to continue during the operation. Similarly, solution is introduced into the check cell by opening cock 52, cocks 49, 48 and 55 being closed, and into the gas-escape cell by opening cock 55, cocks 49, 52 and 48 being closed. About a cubic centimeter of solution is used in each of the last two cells, but measurement is unnecessary.

In operation, the solution in the indicator cell darkens and must be renewed with each test. The solution in the check cell and that in the gas-escape cell should not darken at all during a test. Before the beginning of each test the operator should be sure that the indicator solution in the check cell is free from discoloration. The solution in the gas-escape cell may darken during periods when the gas stream is not flowing through the series, but its color is not important. Any slight darkening of the solution in the check cell must be detected immediately, however, for such color changes would indicate that some oxygen is passing the indicator cell. This might occur if the rate of oxygen supply in the gas stream from the selector were too great to be determined. In the studies here reported the gas always flowed from the absorber through the selector and indicator at a rate of about 0.8 cc. per minute and the arrangement here described always gave complete oxygen absorption in the indicator cell (without any color change of the solution in the check). If the oxygen content of the entering gas is too high for this arrangement, no test can be made.

(b) THE COLORIMETRIC TESTS.—Any oxygen contained in the gas stream from the absorber is taken up by the solution in the indicator cell, which gradually changes color, becoming darker as the test proceeds. The rate of this color change is used as a measure of the rate at which the gas stream delivers oxygen to the indicator cell, which is taken as equivalent to the rate of entrance of oxygen into the absorber itself.

To determine the rate of change of color in the indicator solution, two standard-color solutions are employed. Color solution A is pale and matches the color of the indicator solution when a very small amount of oxygen has been absorbed. Color solution B is considerably darker than A and matches the color of the indicator solution when a larger amount of oxygen has been absorbed. These solutions are housed in sealed vials, similar in size and shape to the indicator cell, two such vials being used for each solution. When the color of the indicator solution is being compared with that of standard A, the two vials containing this standard are placed to the right and left of the indicator cell, on the same level with it and as close to it as is convenient. When comparison is being subsequently made with standard B, the vials of standard A are replaced by those of B. Color comparison of the solution in the indicator cell with that of a color standard is made by visual observation with transmitted light from an incandescent electric lamp, the latter enclosed in such a way that all three vials are equally illuminated from behind and are viewed from the front. Equal illumination of the three vials is secured by means of a vertical white-paper diffusion screen between them and the lamp. In front of the vials is a vertical black cardboard guard with a rectangular window about 4 mm. long and 3 mm. high, opposite the middle portion of the mass of solution in each

vial. The middle window is so placed that the observer's eye receives through it only a rectangular beam of light transmitted horizontally through the unobstructed solution in the indicator cell, and the two lateral windows are on the same level, each being about 2 cm. from the middle one. From each lateral window the eye receives a beam of light like that received from the middle window but transmitted through a color standard.

The arrangement of the color standards may be modified by placing standard A to the left and standard B to the right. The former method is the more precise, but the latter method is convenient and is usually employed with test times of 15 minutes or less, it being particularly useful with test times of about four minutes, as in standardizing absorbers.

The source of light used was a "Mazda" lamp of 60 watts capacity, operated with an alternating current of 110 volts, and the standard-color solutions used are based on the characteristics of the radiation emitted by a seasoned lamp of the above-named brand and capacity. If light of other qualities or intensities were used one or both of the standard-color solutions would doubtless need to be modified.

The rate of color change in the solution in the indicator cell is measured as the length of time required for that solution to change from a color that matches standard A to one that matches standard B. In preparing for a test, the gas from the absorber to be studied is first allowed to flow through the indicator for at least 15 minutes, after which the solution in the indicator cell is replaced by a fresh charge from the mixer. The gas stream continues and the observer records the time at which the color of the solution in the indicator cell matches that of the pale standard. Observations are then made at suitably frequent intervals until the solution in the indicator cell is judged to have acquired the same color as that of the darker standard, when the time is again recorded. The length of period required for this definite amount of color change is thus determined. It may be called the *test time*. Obviously, the length of the test time may be considered as reciprocally proportional to the mean rate at which oxygen entered the absorber during the test. Thus, if the test times for two tests on the same absorber differently exposed to oxygen supply were 20 and 30 minutes, the rate of oxygen supply in the second case would be two-thirds as great as in the first.

Without further considerations of standardization, the method as thus far described suffices for comparing the oxygen-supplying powers of different exposures of the same absorber, provided that the absorber is capable of delivering oxygen to the gas stream as rapidly as oxygen is delivered by the surroundings to the external absorbing surface of the absorber, and also provided that the rate of delivery of oxygen through the absorber wall

to the gas stream is not so great as to produce color change in the check cell or to give a test time too short for satisfactory determination. When the test time is less than 15 minutes the indicator cell should be agitated at every observation and for very short times it should be continually agitated throughout the test. For such short test times, even with continuous agitation of the indicator cell throughout the test, the color change of the indicator solution is apt to be more or less premature or prepunctual. No difficulty has been experienced with the determination of the beginning of the test-time period, but the determination of its end requires special care when the test time is less than 20 minutes. In such cases the color of the indicator solution may be observed to match that of color standard A and, after the gas flow has been stopped, the color change may be reversed and the indicator solution may become somewhat paler. This indicates that the test time recorded is shorter than it should be. It is therefore desirable to bear in mind this prepunctuality of the color change when test times of 15 or 20 minutes, or less, are encountered. When the color end-point is reached the gas flow is stopped and agitation of the indicator cell is continued for a few minutes. If the indicator solution becomes paler the gas flow is resumed for a minute and the treatment is repeated, till the indicator solution maintains its agreement in color with standard B for five minutes after the gas flow has ceased. The exact explanation of the phenomenon here called prepunctuality has not been worked out. Perhaps the method may be improved in some way so as to avoid this phenomenon altogether. At worst, however, it makes necessary a little special care with test times that approach the limit of the method. It really introduces no serious difficulty in the actual use of the method, for the test times for different absorber exposures at the same depth and in the same soil mass are apt to vary considerably, because of the group of unknown reasons usually called chance. It seems safe to say that observed test times of 15 minutes or less are, even without special precautions, not to be corrected by increments of more than 7 or 8 minutes. Thus, an observed test time might be 15 minutes and the requisite correction might increase this to less than 25 minutes. A correction graph may easily be worked out, but such refinement is hardly needed in the present stage of the study of environmental oxygen-supplying power. Any test time less than an hour or two is to be considered simply as short, indicating high oxygen supplying powers of the environment in question.

To renew the solution in the indicator cell the used solution is discarded and a fresh charge is introduced from the mixer at the same time. The mixer connections are shown by the diagram of fig. 9. Cock 51 (fig. 8) is closed and cocks 49 and 50 are partially opened simultaneously. The ad-

justment of these cocks is so accomplished that the level of the solution meniscus in the indicator cell is kept practically constant during the operation. As fresh solution enters, the used solution is swept out through the waste conduit. When the solution in the indicator cell appears to be definitely paler than color-standard A, cocks 49 to 50 are closed and cock 51 is opened. The solution is then brought to the proper level by slightly opening cock 49, if more solution is to be added, or by slightly opening cock 51 if some solution is to be withdrawn, the proper level being determined by means of the two marks on the indicator vial as mentioned previously.

Whenever the indicator solution has been allowed to become much darker than the darker color standard, and tests are to be resumed, the indicator cell is recharged twice in immediate succession. The first new charge is allowed to stand for a period of about ten minutes, with the gas stream to be tested continuously directed through the cell. The cell is then recharged again and tests may be resumed.

(c) APPARATUS FOR PREPARING AND DELIVERING THE INDICATOR SOLUTION.—The indicator solution is an aqueous solution of pyrogallol and potassium hydroxide, the volume-molecular concentration of the former being 0.15 and that of the latter 2.0. A stock solution of each is first prepared and then proper amounts of these are brought together under nitrogen in the mixer. (The arrangements of the mixer and related parts of the apparatus are shown diagrammatically in fig. 9). The mixture is then properly diluted by the introduction of de-oxygenated distilled water. The mixer is connected with the liquid conduit that supplies the indicator cell, the check cell, the gas-escape cell and the telltale, so that indicator solution may be supplied to each of these separately.

With reference to fig. 9, the reservoir for oxygen-free distilled water is connected (1) with a burette for measuring water to be passed into the mixer, (2) with another burette for measuring water to be passed into the receptacle for the preparation of stock pyrogallol solution, (3) with the distilled water supply, (4) with the de-oxygenated illuminating gas supply and (5) with the aspirator system. The several burettes connected with the reservoir are filled from it by gravity. Water is drawn into the reservoir, from the distilled water supply, through cock 39, suction being applied from the aspirator system through cock 46, while all other cocks (34, 44 and 40) connected with the water reservoir are closed. When the desired amount of water has passed into the reservoir, the water supply is cut off (cock 39), and the water in the reservoir is next practically freed from oxygen by permitting de-oxygenated illuminating gas (from cock 40) to bubble through it and pass out through the aspirator system. De-oxygenated nitrogen might have been employed but illuminating gas was used in this

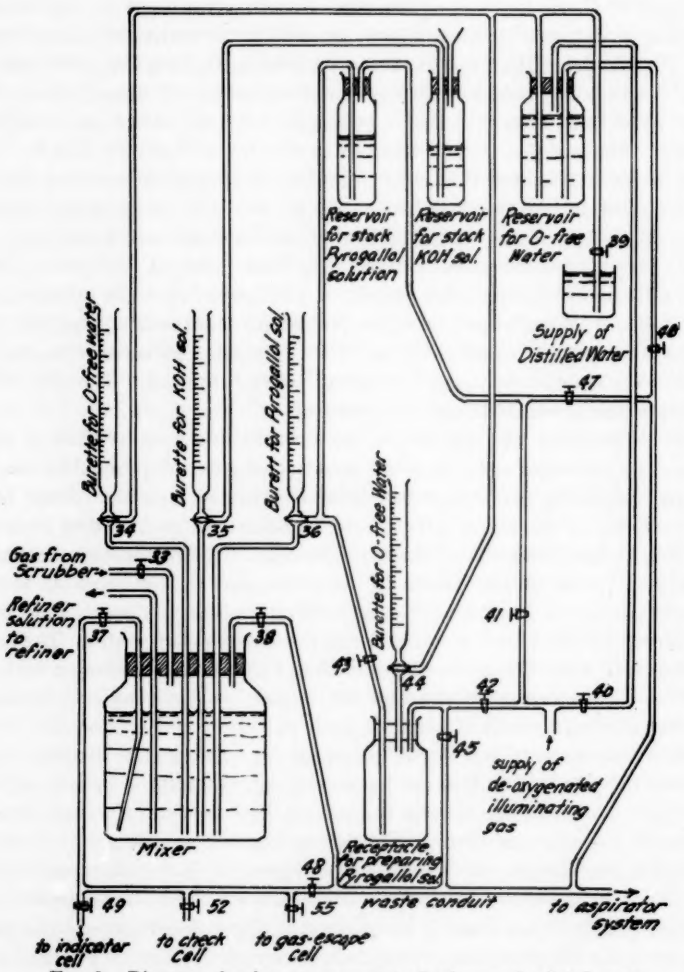

FIG. 9. Diagram showing arrangement of mixer and related parts.

work. De-oxygenated illuminating gas for this purpose is prepared by passing gas from the regular laboratory supply through several scrubber bottles arranged similarly to those used in connection with the nitrogen supply. In this case, however, because of lower pressure from the supply, the gas is passed through the chamber of each scrubber bottle without bubbling through the solution. A half hour of bubbling in the reservoir,

at the rate of 3 or 4 bubbles per second, the tube orifice being about 5 mm. in diameter, renders a liter of water sufficiently free from oxygen for subsequent use in the mixer. To start the siphons supplying de-oxygenated water to the two water burettes it is of course necessary to apply a little suction temporarily to each burette, letting the suction cease as soon as the siphon begins to operate.

Pyrogallol suitable for the indicator solution should not produce noticeable turbidity in the pyrogallol solution, nor should it produce in the completed indicator solution any color more intense than that of the palest standard-color solution to be employed. Powers-Weightman-Rosengarten Co.'s "Analytical" pyrogallol has been found to be satisfactory. Of course, commercial grades are used for the scrubber solution. Pyrogallol for indicator solution for the whole investigation should be of the same lot and the entire supply should be thoroughly mixed and then weighed into 50-gram charges, each hermetically sealed.

Stock pyrogallol solution is prepared in the receptacle therefor (a 500 cc. bottle), the stopper of which is fixed in position by its attachment to the three tubes that it bears. One of these tubes is connected with the reservoir for storing stock pyrogallol solution, another is connected with the burette for introducing measured quantities of de-oxygenated distilled water, and the third is connected with the aspirator system and also with the de-oxygenated illuminating gas supply. The reservoir for storing stock pyrogallol solution bears two tubes, one connected with (1) the de-oxygenated illuminating gas supply and (2) with the aspirator system, and the other with (1) a burette for measuring stock pyrogallol solution to be introduced into the mixer and (2) the receptacle in which the stock solution is prepared. Before stock pyrogallol solution is introduced into its storage reservoir, the air in the reservoir and connecting tubes is displaced by de-oxygenated illuminating gas. To accomplish this, cocks 41, 43 and 45 are opened, the air being drawn off through the aspirator system.

Preparation of stock pyrogallol solution proceeds as follows. The bottle receptacle therefor is removed from its stopper and set aside. Any water contained in the water burette leading into the receptacle is allowed to escape and the water that has stood in the tube leading from water reservoir to burette is allowed to be displaced by fresh water from the reservoir. Also, the air in the reservoir for stock pyrogallol solution is to be displaced by de-oxygenated illuminating gas, which is done by opening cock 41 for a time; gas from the reservoir is allowed to escape at the lower end of the tube that joins the lower receptacle with the reservoir above. Cock 43 is open during this process and is afterwards closed.

A charge (50 grams) of pyrogallol crystals is next introduced into the bottle receptacle for preparing pyrogallol solution and the receptacle is then

returned to its stopper. Air in the receptacle is next mostly removed through the aspirator system by temporarily opening cock 45. One-hundred cc. of de-oxygenated water is now added from the water burette, which has previously been freshly filled from the water reservoir (3-way cock no. 44). While the crystals are dissolving, the receptacle may be slightly agitated, which is possible on account of flexible rubber connections, and the process is hastened if a vessel of warm water is placed under the receptacle and then raised until the lower part of the latter is submerged. The partial vacuum over the pyrogallol solution in the receptacle is next satisfied by allowing the entrance of de-oxygenated illuminating gas through cock 42, which is then again closed. After this the newly prepared charge of stock pyrogallol solution is transferred to the reservoir for stock pyrogallol solution above. To do this, suction is applied in the reservoir by means of the aspirator system, through cock 47. Cock 43 is likewise open during this process, as is also cock 42, which allows the solution leaving the receptacle to be displaced by de-oxygenated illuminating gas. Cocks 42, 43 and 47 are now closed and cock 41 is opened to allow illuminating gas to satisfy the partial vacuum over the solution in the upper reservoir. The last mentioned cock is left open.

Aqueous pyrogallol gradually darkens somewhat on standing, but only slowly under the conditions provided in the reservoir for stock pyrogallol solution. The solution may stand in the reservoir for about two weeks, but when more than two weeks old it is generally best to discard what is left in the reservoir and replenish with fresh solution. To discard the contents of the reservoir, the receptacle bottle is removed from its stopper, cock no. 43 is opened, and the solution flows out by gravity through the same tube as that by which it was passed from the receptacle to the reservoir.

Potassium hydroxide solution is prepared in an open beaker by dissolving one pound of sticks in 500 cc. of distilled water. The purest product obtainable should be used for this purpose, with special precaution that it is free from iron. "Merck's reagent" has been found satisfactory. The solution is poured into the reservoir for stock potassium hydroxide, through a small funnel that penetrates the stopper. This reservoir stopper bears also a short tube of small bore, which serves as air vent, as well as the outlet tube that joins the reservoir with the hydroxide burette. No special arrangement is introduced for emptying the hydroxide reservoir, this being accomplished when necessary by removing the reservoir from its stopper. The solution may stand in the reservoir for a month or more without injury, since the openings to the atmosphere allow only very slow entrance of carbon dioxide and very little movement of water vapor. Experience indicates that this solution does not need to be guarded from atmospheric oxygen.

(d) PREPARATION OF THE INDICATOR SOLUTION.—To prepare an initial charge of indicator solution, air is first displaced from the mixer and its connecting tubes (see fig. 9). Some liquid is introduced into each of the 50 cc. burettes, which are connected with the mixer, from the corresponding reservoir. The siphons may be started by suction as mentioned above; the cocks 34, 35 and 36 are successively opened, and the tube leading into the mixer from each burette is filled, a small amount being allowed to enter the mixer itself.

Aspiration is next applied to the mixer through cock 38 and the mixer is then completely filled with water by successively filling and emptying the water burette, accomplished by manipulating the 3-way cock (34) at its base. Cock 38 is now closed and cock 37 is opened, so that the water just placed in the mixer is removed through the aspirator, refined nitrogen being simultaneously admitted through cock 33. This cock is then closed and the mixer is next rinsed by introducing and removing a small preliminary amount of the regular indicator solution. This is done by introducing into the mixer from the proper burettes, in the order given, 3 cc. of stock pyrogallol solution, 5 cc. of stock potassium hydroxide solution and 50 cc. of de-oxygenated water. While these solutions are being introduced, suction is continuously applied to the mixer through cock 38. This preliminary rinsing solution is then removed by the aspirator through cock 37, being replaced by refined nitrogen delivered through cock 33. One rinsing is found to be sufficient. Cock 33 is again closed and the regular charge of indicator solution is now prepared in the mixer just as was the rinsing solution, the amount used being 18 cc. of stock pyrogallol solution, 30 cc. of stock potassium hydroxide solution and 300 cc. of water. Finally the nitrogen cock 33 is opened. A freshly prepared charge of indicator solution should be allowed to stand in the mixer for a half hour or longer to insure complete mixing.

When the apparatus has been in operation and there is some old indicator solution in the mixer, that residue is of course first removed, just as was the water and afterwards the rinsing solution. While this is being done the burette cocks for the two solutions are manipulated so as to allow all solution that has been standing in the burettes or in the tubes leading to or from the latter to pass into the mixer. The mixer is next rinsed *at least three times* with small preliminary charges of indicator solution, in the manner described above, and the new charge of indicator solution is then prepared.

(e) THE COLOR STANDARDS.—As has been said, two different color-standard solutions are used in the colorimetric tests, one of a pale color corresponding to the color of the indicator solution at the beginning of the

test period and the other of a darker color corresponding to the color of the indicator solution at the end of the test period. In the present section these two standard-color solutions will be described, the pale one being called standard-color solution A and the darker one being called B.

The color-standard solutions are made by bringing together in acidulated water the proper proportions of the three colored salts, cobalt chloride, cupric chloride, and ferric chloride (**2**). The cobalt chloride used was of the Baker and Adamson Chemical Company's "Standard Purity, C. P." The other two salts were "Baker's Analyzed Chemicals," from the J. T. Baker Chemical Company. For dissolving them and for diluting the solutions acid water was used, this being prepared by adding hydrochloric acid solution having a specific gravity of 1.172 to distilled water in the proportions of 1 cc. of the acid solution to 99 cc. of water. A stock solution of each salt was first prepared by dissolving 50 gm. of the material from the original container and making up to 500 cc. of solution.

The exact composition of each of the two standard-color solutions is shown in table I. It is to be noticed that solution B contains no ferric chloride.

TABLE I

COMPOSITION OF STANDARD-COLOR SOLUTIONS

COMPONENTS	COLOR-STANDARD A	COLOR-STANDARD B
Acid water	50 cc.	50 cc.
Stock solution of cobalt chloride	24 cc.	100 cc.
Stock solution of cupric chloride	8 cc.	20 cc.
Stock solution of ferric chloride	2 cc.	.....

(f) COLOR OF THE INDICATOR SOLUTION.—In these studies pyrogallol described as containing no gallic acid was used for preparing the indicator solution, and, as already mentioned, the color standards A and B match respectively a light and a dark shade of this solution as it changes color with absorption of oxygen from the gas stream. The fresh indicator solution, as viewed in the indicator cell before any oxygen has been absorbed, is almost colorless. With the absorption of oxygen its color alters through light orange-browns (a stage of this region being matched by standard A), darker browns, reddish browns (a stage of this region being matched by standard B), wine red, and finally becomes nearly black. If the indicator solution fails to match either of the standards at the corresponding stage of its color change, this may be due to impure materials used in preparing the indicator solution itself or to substances acquired from the rubber tubing connections. In the last case the indicator solution may assume purplish tints as it ab-

sorbs oxygen. It is convenient to control the liquid inlet of the indicator cell with a Hoffman tubing-clamp on a rubber tubing connection, and the possibility of solution contamination from this source may be entirely obviated (1) by using tubing that has been dipped in hot paraffin, so that the solution does not come directly in contact with the rubber, and (2) by allowing solution to flow through the indicator cell, in recharging the latter, until none remains that has stood longer than 20 minutes in the connection. Of course the rubber connection might be replaced by glass tubing and a glass cock, but glass cocks are apt to be troublesome, when used for alkaline solutions.

5. Additional notes on the method and apparatus

A few disconnected, additional notes on operation are here given, which might not be so readily understood if they had been inserted in the preceding sections.

Calibration of the absorbers and interpretation of index values.—As has been said above, as long as only a single absorber is used it is possible to make comparisons between the oxygen-supplying powers of the soil at different depths, etc., without considering the area of the absorbing surface of the absorber, for that surface then remains the same for all tests. Even under these conditions it must be remembered, however, that no test can be made for any absorber environment unless the absorber is capable of taking in oxygen through its porous wall just as rapidly as that substance is supplied by the environments to the outer surface. When exposed in environments of very high oxygen-supplying power (as the ordinary atmosphere, for example), any absorber always gives the same test time, which is really a measure of the ability of the absorber wall to transmit to the gas stream, oxygen that arrives at its external surface. The water-impregnated, porous-porcelain absorbers used in these studies all give test periods of less than 4 minutes when exposed to the ordinary atmosphere. There is no doubt that an absorber so exposed receives oxygen on its outer surface much more rapidly than is indicated by this short test time and it is clear that the length of this test time is not at all a measure of the oxygen-supplying power of ordinary air. It follows from this that, in seeking to determine the oxygen-supplying powers with different absorber exposures in the soil, etc., no account is to be taken of determinations with this type of absorber unless the test times are more than 4 minutes. When the test time is greater than 4 minutes it is indicated that the environment is not supplying oxygen to the outside of the absorber as rapidly as the absorber might deliver it to the gas stream if the rate of supply were greater. The environmental supplying power is, in such

cases, the limiting condition that determines the length of the test time, for we are sure that the absorbing power of the absorber is not taxed under these conditions. As long as this threshold rate of operation for the absorber itself is not reached or too closely approached, the various degrees of oxygen-supplying power of the several environments are to be considered as inversely proportional to the lengths of the corresponding test times.

Of course it is desirable that determinations made with different absorbers should be comparable, and this necessitates calibration of all absorbers with reference to their absorbing surfaces. In the work here reported, as many as eight absorbers have been employed at once, with the gas flowing continuously through all of them, although determinations for some of them were made only at long intervals. The employment of several absorbers, whether simultaneously or not, immediately introduces the requirement for absorber-standardization or calibration; for no two absorbers may be expected to have exactly the same ability to deliver oxygen to the gas stream under any given set of environmental conditions. Absorbers may differ with respect to this ability in two ways. (1) The amount of surface exposed to the environment may be larger for some than for others. (2) Absorbers may differ with respect to average thickness of wall, etc., that is, with respect to the average permeability to diffusing dissolved oxygen. As has been said, all absorber cones used in these studies were so prepared that they gave, when exposed to the ordinary atmosphere at room temperatures, test times of less than 4 minutes and the external surfaces exposed were nearly alike. Since the oxygen-supplying powers of the environments to be tested were practically always such as to give test times of more than the 4-minute limit, no attempt has yet been made to secure calibration coefficients with respect to the average permeability of the absorber walls. The area of the absorbing surface exposed by each absorber was carefully determined, however, by measurement and calculation. The results of these calibrations were always used when determinations secured by means of different absorbers were to be compared.

During the progress of these studies absorbers have remained undisturbed in various soil environments, in the laboratory and out-of-doors, for periods of a year or more and none have given any evidence of sufficient alteration of the absorber wall to impair the ability of the latter to operate properly. All absorbers tested in air after long exposure to the soil have supplied oxygen to the gas stream at a sufficiently rapid rate to give the standardization test time of four minutes or less.

To be acceptable in this work an absorber must have given in ordinary atmosphere a test time less than 4 minutes, as has been said. When the cones are in operation in environments that are to be compared with respect

to their oxygen-supplying powers, the test time for each determination is first to be compared with its calibration test time in air. If the former is not greater than the latter the determination in question is regarded as beyond the capacity of the apparatus; all that can be said is that the oxygen-supplying power of the environment thus tested is about equal to or greater than the absorbing capacity of the instrument. In soil tests this result can be secured only with extremely well aerated soils and with very shallow depths, and the oxygen-supplying power of the soil in such cases may be safely considered as ample for the health of any plant. It is soil environments of low oxygen-supplying powers that are mainly interesting when determinations of these powers are being compared.

Test times for different absorbers in actual determinations may not be directly compared, even when each of them is greater than the corresponding calibration test time, since the external areas of the absorbing walls are generally not exactly alike. Nor can the reciprocals of the determination test times be directly compared if secured with different absorbers. These reciprocals may be termed indices of oxygen-supplying power. It is necessary, before comparing them, to correct each one by dividing by the actual area of the absorbing cone with which it is secured. The corrected indices, which are directly comparable, are denoted by the formula $\frac{1}{ta}$, where t is the test time in minutes and a is the area in sq. cm. of the corresponding absorber.

To illustrate these points, an absorber with an external porous surface of 64.0 sq. cm. (a) gives in a certain soil environment a test time of 70 minutes (t) and the corrected index of the oxygen-supplying power of the given environment is consequently $\frac{1}{64\times 70}$, or $\frac{1}{4480}$; while another absorber with an area of 65.64 sq. cm. (a') gives a test time of 160 minutes (t') and the corrected index for the second environment is consequently $\frac{1}{65.64\times 160}$, or $\frac{1}{10502.4}$. The oxygen-supplying power of the first environment is shown to be 134 per cent. greater than that of the second. Both t and t' must be greater than 4 minutes with the absorbers used in the work here reported.

For simply comparative purposes it is not necessary to express the indices in terms of ordinary units of oxygen; any environment may be compared with any other after the manner of the illustration just given, and the oxygen-supplying powers of any number of environments may each be expressed in terms of that for some selected environment, the latter considered as unity. In physiological investigations it may, however, be desirable to express the index of oxygen-supplying power as a number of milli-

grams of oxygen that might be delivered per hour through, or across, a square meter of absorbing surface. To accomplish this it is necessary to determine the exact value, in milligrams, of the oxygen unit (u) of the colorimetric determination. By several tests, the oxygen unit of the present studies was found to be approximately 0.03 mg. That is, the solution in the indicator cell absorbed 0.03 mg. of oxygen while changing in color from that of color standard A to that of standard B. The value of u would of course be different if the indicator solution were different from the one here used or if it were employed in different amount; or if either of the color-standard solutions were different from the corresponding one described above.

Turning again to the general formula for the relative index value, it is seen that, for any determination, u mg. of oxygen is supplied in t minutes through a surface of a sq. cm., a rate of supply equivalent to $\frac{u}{ta}$ mg. per minute per square centimeter of absorbing surface. Through the surface of a square meter the corresponding maximum supplying power of the given environment would be $\frac{10{,}000\,u}{ta}$ mg. per minute, and that of a square meter of surface would be $\frac{600{,}000\,u}{ta}$ mg. per hour. In the illustration given above, the first environment can deliver $600{,}000 \times 0.03 \times \frac{1}{4480}$, or 4.02 mg. of oxygen per square meter per hour $\left(\frac{600{,}000\,u}{ta}\right)$ and the second can deliver $600{,}000 \times 0.03 \times \frac{1}{10502.4}$, or 1.71 mg. $\left(\frac{600{,}000\,u}{t'a'}\right)$. To express any index value in terms of milligrams per hour per square meter of surface, we simply multiply the relative index value $\left(\frac{1}{ta}\right)$ by $600{,}000\,u$; or, since the value u is 0.03 mg., by 18,000; we thus change the expression from colorimetric units of oxygen per square centimeter per minute, to milligrams of oxygen per square meter per hour. In reporting the experimental results below, the index values will be given in terms of milligrams of oxygen per square meter per hour.

TESTING THE APPARATUS BEFORE INTRODUCING SOLUTION.—The complexity of the apparatus makes it desirable to test its operation after all parts have been assembled, using distilled water instead of solution. After it has been observed that the pressure and orifice controls are functioning properly, and no difficulty is experienced in any of the necessary manipulations, the various solutions may be introduced and actual operation may be undertaken.

ADJUSTMENT OF THE THROTTLE-CONTROL.—Occasionally it becomes necessary to draw off a liter or more of nitrogen from the scrubber while the apparatus is in operation, as in recharging the refiner tubes with fresh solution or in discarding aged indicator solution from the mixer. This may be done in the following manner without seriously altering the established gas pressures in the scrubbers. The rate of bubbling of nitrogen as it enters the first scrubber unit is determined, then the throttle-control valve is opened sufficiently to allow gas to escape freely from the secondary pressure-regulator. Nitrogen is then passed into the part of the apparatus where needed, care being taken that the movement is not rapid enough to reduce the gas pressure in the scrubber below the point where gas ceases to escape from the pressure control. After this has been accomplished the normal rate of flow of gas from the pressure cylinder is re-established by again stopping down the throttle-control valve until the former rate of bubbling in the first scrubber unit is resumed.

USE OF THE ASPIRATOR SYSTEM.—For many purposes gravity and the gas pressure in the different containers are sufficient to move the solutions from one part of the apparatus to another as desired, and it is generally better to employ these forces when they suffice than to use suction through the aspirator, which is apt to be uneven and sometimes violent. For example, it is best not to use the aspirator for removing and introducing solution in operating the cells of the indicator system. As has been indicated, the aspirator is necessary in many cases, however, but when it is used care should be exercised not to allow pressure gradients to become unnecessarily great. Excessive pressure differences may strain the apparatus at many points; air may be caused to enter through glass cocks, that are otherwise tightly closed, mercury may be drawn from the mercury seals into the tubes, etc. If aspirator suction is ever applied to the indicator cell, care must be taken that mercury is not drawn from the selector up into the lead tube leading to the indicator cell; a brief contact of mercury with the tube wall would ruin the tube by amalgamation. Other similar precautions might be mentioned, but the operator who has assembled the apparatus will be familiar with its weaker and stronger points and will be guided by that familiarity.

RUBBER-TUBING CONNECTIONS.—While glass or metal cocks might well be used throughout the apparatus, ordinary rubber connections with Hoffman tubing clamps have been found to serve well except where glass cocks have been specified. The pieces of rubber tubing should first be dipped in hot melted paraffin, which greatly increases the imperviousness of their walls to oxygen diffusion. For the burettes connected with the mixer system it is advisable to use clamp-controlled rubber connections between each burette

and its supply reservoir and between the burette and the mixer. Each burette is furnished with the usual 3-way glass cock, however, and when a burette is to be used the proper clamps are first opened, allowing the flow of liquid to be controlled by the three-way cock. When the burette is not in use the clamps are kept closed, to prevent any possibility of leakage past the cock. Furthermore, it is occasionally necessary to remove the plug of the glass cock for cleaning or greasing, and the closed clamps make this possible without risk of air being admitted to the tubes or of solution being wasted.

6. General applicability of the new method

While the method described in the preceding pages has been worked out for the purpose of comparing soil environments as they may influence plant growth, it should be suitable for studies of any environments that supply oxygen to an oxygen-absorbing surface at slow rates. Modification of the apparatus here described may doubtless make it available for studies of environments that offer more rapid rates of oxygen supply. Also, the apparatus and general method here employed should, with suitable changes, be useful for studying supplying powers with reference to other gases, such as carbon dioxide.

IV. Experimentation

1. Experiments with soils

Some experimental tests of the oxygen-supplying powers of soils have been carried out, partly with the apparatus described in the preceding section and partly with the preliminary form described by Hutchins and Livingston. The results of these tests are presented below as experiments numbered from 1 to 6, inclusive. The first three experiments were carried out with the original apparatus, the standardization of which was not as nearly perfect as is that of the improved apparatus described in the present paper. The remaining experiments were performed with the new apparatus. Of the first three, the data of number 1 were used in the preliminary publication on this subject by Hutchins and Livingston. Experiments 2 and 3 were performed with the earlier apparatus but are now recorded for the first time. Since no tests were made to compare the oxygen-absorbing efficiency of the two forms of apparatus, it is best in the case of these three experiments to neglect the actual numerical values, as published for the present experiment 1 in the Hutchins and Livingston paper, regarding them merely as indications of relative magnitudes and without attempting detailed quantitative comparisons between them and the

corresponding values secured by means of the improved apparatus. For experiments 4, 5 and 6 of the present series, the quantitative values based on the more nearly perfect standardization of the improved apparatus will be considered.

EXPERIMENT 1.—The first soil experiment, as mentioned above, has been somewhat fully described in the preliminary paper on this subject. An outline of the essential features and results of this experiment follows. A single, previously tested absorber was placed in a box of loosely sifted, moist soil, 8 cm. from the bottom and 7.5 cm. from the top. During the first two days of the experiment, the oxygen-supplying power indicated by the absorber gradually decreased and was then maintained with but negligible fluctuation for over forty days. An additional 8 cm. layer of loosely sifted, moist soil was then added to the box, thus bringing the absorber to a depth of 16 cm. below the soil surface. A lengthening of the test time was evident after three hours and continued during the next three days, after which the oxygen-supplying power was shown to be about 76 per cent. of the original maintained value just mentioned. Water was next added to the soil, corresponding to 6 cm. of rainfall and nearly saturating the soil. The resulting soil shrinkage lowered the surface so that the absorber was now only 13.5 cm. below the surface. Eight hours later the supplying power was found to be about 46 per cent. of the original value and the same continued to hold for 18 hours. Then the soil was firmly packed, thus bringing the absorber to within 11 cm. of the soil surface. Immediately after packing had been completed, an oxygen-supplying power 32 per cent. as great as the original was indicated, and another determination beginning four hours after the packing and lasting 84 hours gave a supplying power only 2 per cent. as great as the original.

If the index of oxygen-supplying power for a depth of 8 cm. in loose, moist soil is taken as 100, then the corresponding power for a depth of 13.5 cm. of firmly packed, nearly saturated soil, was only 76. Adding 8 cm. of loosely sifted soil diminished the relative value of the oxygen-supplying power at the absorber surface to 46. The latter value was still further decreased to 32 when the soil was thoroughly wetted, and to 2 when it was wetted and firmly packed.

EXPERIMENT 2.—In the second soil experiment an absorber was centrally placed in moist soil held in an ordinary porous flower pot of the 9-inch size, with the roots of a healthy *Pelargonium* plant lying in the immediate neighborhood of the absorber surface. The oxygen-supplying power of the soil mass was studied in relation to alternate wetting and drying of the soil. The absorber used was of the conical type, but somewhat larger and with thicker walls than are specified in the description of the apparatus

given in earlier parts of this paper. Twenty-four hours after the beginning of the experiment the index of oxygen-supplying power was determined. The pot was then thoroughly watered from above in the ordinary way and was placed with its base in water to a depth of 2 cm. After 17 hours in this position the index value was only 42 per cent. as great as in the original determination. The pot was then removed from the water, and drainage and evaporation were allowed to proceed freely for 30 hours, during which period tests were frequently made, the index value gradually increasing to 89 per cent. of the original. The soil mass was next thoroughly saturated by temporarily plunging the pot into water, and the pot was then allowed to drain with its base standing in a 2 cm. water layer. When drainage had ceased the index value was found to be 53 per cent. of the original. The preparation was then removed from the water and freely exposed to the air for 18 hours, at the end of which period the index had risen to 80 per cent. of the original. The soil was next packed enough to lower its surface in the pot about 2 cm., which of course increased the soil water content with reference to the soil volume. A test 15 hours later gave an index value of 47 per cent. of the original. For the next 50 hours evaporation was allowed to proceed, the water content of the soil gradually decreasing and the index value gradually increasing, until the latter became once more 80 per cent. as great as the original value.

EXPERIMENT 3.—The third soil experiment was similar to the second, with a 9-inch flower pot of garden soil and a centrally placed absorber, but without any plant. Beginning with moist, loosely packed soil, the index of oxygen-supplying power was determined five hours after starting the experiment. The pot was next stood with its base in water to a depth of 2 cm. for 44 hours, during which period the index of the oxygen-supplying power of the soil at the surface of the absorber decreased gradually, as shown by frequent tests, to 15 per cent. of the original determination. The pot was then removed from the water and a strong air current from an electric fan was directed against the pot wall for 40 hours, to hasten evaporation. During this 40-hour period the index of the oxygen-supplying power of the soil steadily increased and at the end of the period it was 88 per cent. as great as the original. The fan was then stopped and the pot was thoroughly watered in the usual way, being again placed with its base in water. Frequent subsequent tests indicated a rapid decrease in the oxygen-supplying power, and a final test after 24 hours gave an index value of 13 per cent. as great as the original value.

The second and third soil experiments show in a general way what may be expected to occur in the case of an ordinary potted plant in a greenhouse or living room, when thoroughly watered once or twice a day. When the

soil of such a pot is only ordinarily moist the oxygen-supplying power of the root environment may be from 2 to 6 times as great as it is immediately after a thorough watering, in spite of the fact that the water added contains much dissolved oxygen.* The index value fluctuates greatly with alterations in the soil moisture content, and it is greater when the soil is loose and less when the soil is more thoroughly packed. It seems probable that ordinary pot-grown plants would be more vigorous if the oxygen-supplying power of the soil might be maintained as high as possible, without allowing the water supply to the roots to become inadequate. To accomplish this it might be desirable to add water to the pot in small amounts and at frequent intervals, but the soil condition here thought of can probably be well maintained in such cases by some automatic water-supplying device such as the LIVINGSTON (**11**) porous-porcelain auto-irrigator.

EXPERIMENT 4.—The fourth soil experiment, and the fifth and sixth also, were carried out with the improved apparatus as has been mentioned, and also with a much larger soil mass than was used in the three cases already considered. A moist, sifted garden soil, similar to that used in the previous experiments, was used. It was held, with medium packing, in an upright, galvanized, sheet-iron cylinder 60 cm. high and 40 cm. in diameter, tight excepting that the top could be opened and that there were three small holes through the wall close to the bottom, these also being opened or closed according to need. Several absorbers were placed in the vertical axis of the cylinder at different depths. The two lead tubes for each absorber were led spirally outward through the soil to the wall of the cylinder and then upward, this precaution being taken to avoid gas interchange with the air above, along the outer surface of the tubes.

With alterations in the packing and moisture content of the soil mass it was found, as is generally true with such cylinders of soil, that a considerable annular air space soon developed just within the cylinder wall, especially near the top. Such separation of the soil from the container wall must of course allow very free air movement in its region, which might bring atmospheric oxygen to the lower absorbers without its having traversed the soil above. To care for this opening was found to be one of the most important problems of technique in such experiments as these. After

* HOWARD and his co-workers (**10**), especially p. 196, have emphasized what may be an important point with reference to precipitation and irrigation, as these are related to soil aeration, namely that rain is generally more beneficial to plants than is flood irrigation of the same amount. The idea is advanced that this may be due to the fact that natural precipitation supplies the soil with water that is highly charged with oxygen, this water percolating to great depths and tending to carry much of its dissolved oxygen with it. These authors have given us much valuable information and discussion on the importance of soil oxygen in agriculture and forestry.

preliminary attempts of various kinds, it was found that this difficulty could be entirely obviated by the introduction of a seal along the surface of contact between soil and cylinder, in the upper region. The best material tried for making this seal was a three-per cent. agar gel containing a small amount of copper sulphate as an antiseptic. In these experiments the cylinder of soil was allowed to stand for several days before the seal was applied, and then the hot liquid agar was simply poured around the edge of the soil surface. Agar was added until downward flow along the wall of the cylinder ceased. The agar solidified within 10 or 12 hours and there resulted a very excellent seal which was found to prevent atmospheric oxygen from finding its way downward at the surface of contact between soil and cylinder. This use of agar gel is in some respects similar to the use of paraffin in the WHITNEY paraffined wire pot for biological tests of soil productiveness (**12, 20**). Agar was found to be more satisfactory than paraffin in the present work.

In the fourth soil experiment four absorbers of the new conical type described in the early part of this paper were used, being placed 10, 20, 30 and 45 cm. below the soil surface. Since, as has been said, the improved apparatus was used in this and the two following experiments, it will be possible to employ the actual values of the supplying power index in reporting the results in all three cases.

The bottom openings of the cylinder were closed in this experiment. After the test time had been determined for each absorber (the index values being, respectively, 25.3, 14.2, 4.3, and 1.7 milligrams per square meter per hour), the top of the cylinder was closed by means of a disk of tinned sheet-iron resting on the soil, with a paraffined seal around the margin. During the next 40 hours frequent determinations were made for each of the four absorbers. The index value of the oxygen-supplying power gradually decreased in every case, until all four index values were below 0.4. Then the top of the cylinder was reopened and determinations were continued during the next three days. The index value for each absorber gradually increased until it attained its initial magnitude.

These results show clearly that the oxygen-supplying power of such a soil mass as that used in this experiment, open at the top but otherwise cut off from the atmosphere, is, as might be expected, relatively high near the soil-air surface and progressively lower as we proceed downward. In this particular experiment the index value for a depth of 45 cm. was found to be only 6.7 per cent. of the corresponding value for a depth of 10 cm. Since all of the oxygen reaching any absorber must have passed through the soil above, the rate of arrival of oxygen at the level of one of the deeper cones must be lower than in the case of a cone nearer the soil sur-

face. To reach the uppermost cone, atmospheric oxygen had to diffuse through only 10 cm. of soil, while it reached the lowest cone only after traversing 45 cm. of soil. The rate of arrival of oxygen at any absorber might therefore be expected to be roughly related to the depth of the cone. As a porous, water-impregnated body, the soil offers resistance to the diffusion of oxygen but it must be remembered that the soil also acts as a reducing agent and that the soil materials and soil organisms are continuously absorbing oxygen, which feature must in effect increase the resistance offered to downward movement of oxygen.

It is interesting to note that closing the top of the cylinder in this experiment rapidly brought all four indices to values much lower than that indicated for the deepest absorber when the top was open. Entrance of oxygen from the air ceased with the application of the upper seal and it is evident that soil processes, such as oxidation and the respiration of organisms, then rapidly used up most of the oxygen that was in the soil at the time of closing.

EXPERIMENT 5.—The fifth soil experiment was performed with a cylinder of soil like that used in the fourth experiment. After the soil had been placed in the cylinder it was thoroughly saturated with water, which was allowed to enter through tubes attached to the three lateral openings near the bottom of the cylinder, the water flowing in slowly and displacing the soil gases until the free water surface appeared above the soil surface. The soil mass was then allowed to drain by opening the three outlets at the bottom. The marginal agar seal was introduced after drainage had ceased, and then the soil surface was covered with a layer of sand to a depth of three centimeters, the sand surface remaining exposed to the air throughout the experiment. Only three absorbers were used in this instance, these being 14, 32, and 42 cm., respectively, below the bottom of the sand layer. Determinations of the oxygen-supplying power were made after the agar seal and sand mulch had been introduced. The index values for the three absorbers, in the order given, were found to be 11.3, 2.3 and 0.08, respectively, and these values were maintained for a period of nine days. An experiment on the germination of wheat seed at different depths was carried out simultaneously with the determination of these index values. The results of this germination experiment will be considered in the next section.

In the case of this wet soil, it is seen that the index values are again lower for greater depths and that the observed differences are more pronounced than in the case of the moist soil of the fourth soil experiment. With the possible exception of the upper few centimeters of the soil mass, it appears that the oxygen-supplying power of the very wet soil for any given depth was much lower than for the same depth in the moist soil of the fourth experiment.

EXPERIMENT 6.—The sixth soil experiment was like the fourth, excepting that the surface of the moist soil was covered with a sand mulch similar to that used in the fifth experiment and the cover used for closing the top of the soil cylinder in the present instance was perforated with a number of holes, 5 mm. in diameter, which were initially closed with wax. No determinations of oxygen-supplying power were made until after the cylinder had been completely closed. As soon as the preparation was complete, however, determinations for the four absorbers were begun and these were continued at intervals for a period of two and one-half days. Then seventeen of the openings in the cover, equally distributed, were opened, admitting air at a limited rate, and determinations were continued seven days longer. Germination tests with a number of different kinds of seeds were carried out in this cylinder of soil, and the results of these will be described in the next section. The index values for critical stages of this experiment are shown in table II.

TABLE II

CHANGES IN INDEX OF OXYGEN-SUPPLYING POWER IN SOIL CYLINDERS WHEN CLOSED AND ON OPENING THEM

DEPTH OF ABSORBER IN CENTIMETERS	INDEX OF OXYGEN-SUPPLYING POWER (MG. PER SQ. M. PER HOUR)				
	Immediately after closing cylinder	1 to 2 days after closing cylinder	2 to 2.5 days after closing cylinder	4 days after opening cylinder	7 days after opening cylinder
10	>75.0	0.9	<0.2	15.4	22.6
20	>75.0	0.9	—	6.2	7.4
30	>75.0	<0.2	—	5.6	2.3
45	>75.0	<0.2	<0.2	2.0	0.5

Immediately after sealing, all absorbers indicated index values of at least 75.0. When the cylinder had been closed for from one to two days all index values had decreased to 0.9 or lower, and during the following half-day the upper and lower absorbers both showed clear indications that their index values were less than 0.2. In the two and one-half day period during which no oxygen was admitted from without, the soil processes and germinating seeds had apparently removed almost all of the oxygen initially enclosed, and the oxygen-supplying power of the soil was consequently very low for all depths.

Removing the seals from the openings in the cover was of course followed by rapid entrance of oxygen from the atmosphere, and after the cylinder had been open for four days the index values ranged between 15.4 and 2.0, as shown in the table. In the following three days the index for the upper absorber increased from 15.4 to 22.6 and that for the second also increased slightly (from 6.2 to 7.4). On the other hand, the indices for the lowest two absorbers decreased during the same three days, from 5.6 to 2.3 for the third absorber and from 2.0 to 0.5 for the fourth. The reversal noted for the two lower absorbers is probably due to germination of the seeds in the soil above them and to the consequent rapid increase in the absorption of oxygen by seedling respiration in the upper soil layers.

SUMMARY OF SOIL EXPERIMENTS.—For the soils used in these tests, when only the upper surface of the soil mass was exposed to the atmosphere, as is generally true for soils in the field, the oxygen-supplying power is shown to be greater near the air-soil surface and smaller for greater depths. It is also shown to be greater, for the same depth, in dryer or less firmly packed soil and smaller for wetter and more firmly packed soil. Of course the actual values of the supplying-power indices should be different not only with regard to depth, moisture content and firmness of packing, but also with respect to the kind of soil dealt with (whether sandy or loamy, etc.) and with respect to the prevalence of aerobic organisms and of readily oxidizable materials in the soil between the absorber and the air-soil surface. Soil temperature, barometric pressure and other climatic conditions may be expected to be more or less influential.

2. EXPERIMENTS WITH SEED GERMINATION

OXYGEN-SUPPLYING POWER REQUISITE FOR SEED GERMINATION.—The germination of seeds furnishes an excellent example of very rapid and vigorous growth, with correspondingly high respiration rates. It is known that the seeds of some plants are able to germinate with very low rates of elementary oxygen supply, while others fail to germinate at all excepting with an abundant supply of elementary oxygen. On account of the ease with which many kinds of seeds may be tested for germination in different environments, some time was devoted in these studies to some preliminary determinations of the approximate oxygen-supplying powers necessary for germination in a few different kinds of seeds. The few experiments that have been performed along this line will be briefly noted below, simply as illustrations and suggestions. The method and apparatus used for determining oxygen-supplying power in all these experiments were of the improved form described in the earlier part of this paper, and the supplying-power indices dealt with below are all expressed in terms of milligrams per hour per square meter of absorbing surface. These values are comparable with those given above for soil experiments 4, 5 and 6.

GERMINATION EXPERIMENT 1.—In the first germination experiment, seed of *Triticum aestivum* (wheat) was first thoroughly soaked in water for 24 hours at room temperature, and was then packed into a sheet metal cylinder similar to the one used for the experiments with soils described above. As the seed was placed in the cylinder, absorber cones were centrally placed, one above the other, after the manner followed in the soil experiments. Twenty-four hours after the placing of seeds and absorbers, germination had just visibly begun to a depth of about 30 cm. and absorbers at depths of 15 cm. and 30 cm. showed index values of 14.1 and 2.4, respectively. At the end of an additional 24 hours germination was well advanced to a depth of about 15 cm. (with rootlets a centimeter or more long), while seeds at greater depths showed no further germination than they had shown at the end of the first 24 hours. After the second 24 hours the upper cone, 15 cm. deep, gave an index value of 1.5 and the second cone, 30 cm. deep, gave one of less than 0.05. Of course all seeds were well aerated when first placed in the cylinder, but those in the upper region must have absorbed oxygen at a very rapid rate, thus taking up most of the oxygen reaching them, and preventing the deeper seeds from getting oxygen at any considerable rate from the air above. Excepting for the first hour or so after the beginning of the experiment, the seeds nearest the surface of the seed mass naturally received the most rapid supply of oxygen, as is shown by the determinations after the first 24 hours. The avidity with which the actively germinating seeds absorbed oxygen apparently increased very rapidly after that time. During the second 24-hour period all seeds germinated well and rapidly down to a depth that showed, at the end of this second period, a supplying power only about one-seventh of the corresponding supplying power at the end of the first 24 hours. It is safe to say that good germination occurred with supplying powers of more than 1.5 mg. per square meter per hour (starting with 14.1 mg. and ending with 1.5 mg.) during the period. The average index value for the second 24 hours, during which active germination occurred, was about 3.0 for the lower depth at which germination was active, and this average value may be regarded as about the lower limit of oxygen-supplying power that would permit good germination. During the first 24 hours, germination began in the region of the seed mass that had oxygen-supplying powers of less than 2.4 mg. per square meter per hour. This indicates that the lower limit of oxygen-supplying power for the germination of a well-watered wheat seed lies between 3.0 mg. and 2.4 mg.; this lower limit seems surely not to be lower than 1.5. Seeds at greater depths than 30 cm. failed to make any visible beginning of germination, and the index values for these deeper regions of the seed mass were all less than 0.05. Of course these conclusions are based only on this particular experi-

ment; with non-oxygen conditions different from those that obtained in this particular cylinder of seed it may be supposed that the environmental oxygen-supplying power requisite for the rate of germination here considered would have had a value more or less different from the mean index value here given.

GERMINATION EXPERIMENT 2.—As has been mentioned, some seed germination tests were made in connection with soil experiment 5 described above. The results of these tests will now be considered as experiment 2 on seed germination. Seeds of the following plants were used: *Triticum aestivum* (wheat), *Zea* (field maize and Navajo maize), *Oryza sativa* (rice) and *Gossypium hirsutum* (cotton). The unsoaked seeds were planted at depths of 2, 14, 24, 34, and 44 cm., the second, fourth and fifth depths being in the vicinity of the oxygen absorbers used. To facilitate the recovery of the seeds from the soil, one piece of paraffined bobbinet was placed 2 cm. below, and another at an equal distance above, each seed layer. As mentioned in the description of soil experiment 5, the completed preparation was saturated with water, drained and allowed to stand nine days. At the end of that period the seeds were examined. At the 2-cm. depth, seeds of all five kinds had germinated and produced vigorous shoots. At a depth of 14 cm., and with an index of oxygen-supplying power of 11.3, field maize, wheat and rice had produced vigorous shoots, while Navajo maize had produced only small roots and cotton had not visibly germinated. At the 24 cm. depth the oxygen-supplying power was not measured, but it was surely between 11.3 mg. (that for the 14-cm. depth) and 2.3 mg. (that for the 34-cm. depth), probably about 4.5 mg. per square meter per hour. Field maize, wheat and rice at this depth produced small roots only, while Navajo maize and cotton failed to show any germination. At the 34-cm. depth, with an index of oxygen-supplying power of 2.3, as well as at the 44-cm. depth, with an index of 0.08, rice alone germinated.

From these results it appears that the environmental oxygen-supplying power necessary for healthy germination of wheat is greater than 2.3 mg. per square meter per hour, and lower than 4.5 mg. This conclusion agrees very well with that derived from the results of experiment 1, in which this limit was estimated as about 3.0 mg. per square meter per hour. The results of experiment 2 indicate also that the minimum oxygen-supplying power necessary for healthy germination is different for different kinds of seeds. The minimum requirement for field maize seems to be similar to that for wheat, while the minimum requirement for rice is shown to be very much lower, and those for Navajo maize and cotton appear to be considerably higher.

GERMINATION EXPERIMENT 3.—The third germination experiment was carried out in connection with soil experiment 6, described above, and was

so planned that the germination of several kinds of seeds at different depths in the soil mass could be studied in connection with the indices of environmental oxygen-supplying power for those depths. Sixteen kinds of seeds were used, being so placed in the soil that a number of seeds of each kind were at each of the following depths: 2, 10, 20, 30, and 45 cm. The last four depths correspond to the depths of the four oxygen absorbers.

To facilitate final observation, the seeds were planted in soil rather firmly packed in special rectangular chambers measuring 8 cm. long, 2 cm. wide and 1.5 cm. deep, open at the top and closed at the bottom with paraffined bobbinet, the lateral walls being of paraffined cardboard. Each chamber served for four (smaller) or two (larger) kinds of seed.

The cylinder was sealed immediately after the placing of the soil, seed-chambers and absorbers. The indices of oxygen-supplying power for the four absorbers all decreased during the next 60 hours to values below 0.2. Then the perforations in the cover of the cylinder were opened so as to admit oxygen at a limited rate, and the values of the indices for all absorbers increased until the index ranges for the three-day period from the fourth to the seventh day were 15.4–22.6 for the 10-cm. depth, 6.2–7.4 for the 20-cm. depth, 6.6–2.3 for the 30-cm. depth and about 0.5 for the 45-cm. depth. The seeds were not soaked before planting and it may be supposed that they had not taken up sufficient water for germination until after the supplying-power rates indicated by these index values had been established in the soil around them.

Seeds of *Gossypium hirsutum* (cotton), *Brassica oleracea* var. *capitata* (cabbage), *B. nigra* (mustard), *Trifolium repens* (white clover), *T. incarnatum* (crimson clover), *Phleum pratense* (timothy) and *Melilotus alba* (sweet clover) germinated well in damp-chamber tests but failed to show visible germination at any of the depths tested in the soil. Apparently these seeds all require, under the other conditions of this experiment, an oxygen-supplying power of not less than 22.6 mg. per square meter per hour. *Hibiscus esculentus* (okra) seed germinated at depths of 2, 10, and 20 cm. but failed to show germination at depths of 30 and 45 cm., the supplying-power requirement for germination being in these cases apparently greater than 5.6 mg. per square meter per hour. Seeds of *Cucumis melo* (muskmelon), *Medicago sativa* (alfalfa) and *Lycopersicum esculentum* var. *validum* (tomato) germinated well at depths of 2, 10, 20, and 30 cm., the minimum oxygen-supplying power necessary for germination of these seeds being apparently not greater than 2.0 mg. per square meter per hour. Seeds of *Trifolium hybridum* (alsike clover), *Oryza sativa* (rice) and *Zea* (field maize, Navajo maize and popcorn) germinated at all depths tested, the minimum oxygen-supplying power necessary for germi-

nation being apparently considerably less than 2.0 mg. per square meter per hour in these cases.

In general the enlargement of the seedlings, where visible germination occurred, took place less rapidly for seeds at the greater depths and more rapidly for those nearer the soil surface. At the time of observation, for example, wheat shoots from seeds at a depth of 2 cm. were about 21 cm. long, while those from seeds 20 cm. deep were about 10 cm. long and the seeds 45 cm. deep exhibited no shoots but bore roots about 2 cm. long. These observations suggest that the early growth of seedlings may exhibit relations to environmental oxygen-supplying power somewhat similar to, but different in detail from, the corresponding relations indicated for germination, and that shoot growth and root growth may in some cases, at least, manifest different relations to oxygen-supplying power.

GENERAL DISCUSSION OF THE GERMINATION EXPERIMENTS.—It is interesting to note that the results of the third germination experiment are consistent with those of the other experiments on germination as far as indications regarding the oxygen-supplying power requirement for the germination of wheat are concerned. Taking the results of the three experiments together, it may be said that the oxygen-supplying power necessary for the germination of wheat seed when well supplied with water and at room temperature lies within or about the range between 3.0 mg. and 2.3 mg. per square meter per hour. It appears from the results of germination experiments 2 and 3 that field maize has a minimum similar to that of wheat, although observations of the seedlings suggest that the minimum for growth of the former may be somewhat lower. Cotton seed appears to require a much higher rate of oxygen-supplying power (perhaps greater than 22.6 mg. per square meter per hour), while the minimum requirement for the germination of rice seed is clearly very much lower than that for the germination of wheat, surely lower than 0.5 mg. per square meter per hour.

As has been said in the introduction to this paper, very little study has as yet been devoted to the quantitative aspect of the oxygen requirements of plant roots. LIVINGSTON and FREE and also CANNON have published a few notes on the general relations between the health and growth of plants and the oxygen content of the soil about their roots. Their published results are summarized here for the sake of comparison.

LIVINGSTON and FREE (**13**) studied the behavior of the roots of some higher plants in relation to soils in which both the moisture content and the oxygen content were fairly well controlled. They found that the response of the plant to deficiency of oxygen in the soil atmosphere varied widely for different kinds of plants; some species (*Coleus blumei* and *Heliotropium peruvianum*, for example) were very sensitive to a deficiency

of oxygen in the soil atmosphere, while other species (for example, a species of *Salix*, probably *nigra*) gave no evidence of any injurious effects when the soil atmosphere was practically without oxygen. It was noted that cessation of intake of water by the roots, as detected by means of the porous-porcelain auto-irrigator (**14**), was the first sign of injury. With *Coleus* and *Heliotropium* this stopping of water absorption was followed by progressively lessened turgor of the shoot and leaf and finally by wilting and death. The plants could be revived when re-supplied with oxygen before wilting became too extensive or severe.

CANNON has made a prolonged and persistent study of the very difficult and no less important problem of the oxygen requirement of plant roots and has recently shown (**4, 5**) that, for roots growing in sand and with a maintained oxygen content of the soil atmosphere, the minimum oxygen content necessary for active root growth is much higher for higher temperatures than it is for lower temperatures, within the range from 18° to 30° C. This writer's comparisons between growth rates for different oxygen contents of the soil atmosphere are based upon what he terms the "normal" growth elongation rate for the maintained temperature employed. This rate is the one shown by roots of the kind in question growing at the given temperature, and under the non-oxygen conditions of his tests, when the oxygen content of the soil atmosphere is great enough so that any slight increase therein would not result in more rapid growth. In other words, the "normal" growth rate for any temperature is the maximum rate for that temperature and for the other non-oxygen conditions, both internal and external, that prevail in a test. It is clear that for any maintained temperature there must be a minimum oxygen partial pressure in the soil air, and a corresponding minimum in the oxygen-supplying power of the soil about these organs, for this elongation rate in any particular kind of roots. For roots that require atmospheric oxygen for growth there must also be a second minimum partial pressure of oxygen and rate of oxygen supply for any growth at all. For roots that need atmospheric oxygen, slow growth should occur with rates of oxygen supply slightly above the last named minimum, and progressively more rapid growth rates should be exhibited with correspondingly greater rates of oxygen supply until the first mentioned minimum is reached, after which higher rates of oxygen supply should give the growth rates called normal for the given temperature, etc., and for the roots in question.

Illustrations from CANNON's data are shown in table III, for *Zea mays* ("Golden Bantam" sugar corn) and for *Potentilla anserina*, a swamp plant.

From these data it is seen that the minimum oxygen content of the soil atmosphere for "normal" growth at 18° C. is five times as great for

TABLE III

RELATION OF TEMPERATURE AND SOIL-AIR OXYGEN CONTENT TO GROWTH RATE OF ROOTS

SOIL TEMPERATURE IN DEGREES C.	PERCENTAGE OF "NORMAL" GROWTH RATE				
	Potentilla anserina		*Zea mays*		
	1.2 per cent. oxygen in soil air	2.0 per cent. oxygen in soil air	3.0 per cent. oxygen in soil air	3.6 per cent. oxygen in soil air	10.0 per cent. oxygen in soil air
18	25	100	33	66	100
20	.....	.....	20	.....	.....
27	0	33	.....	.....	.....
30	.....	25	6	33	90

maize as it is for *Potentilla,* since the minimum oxygen supply for "normal" growth corresponds to a 10-per-cent. oxygen content in the soil atmosphere for maize and to a 2-per-cent. content for *Potentilla.* Also, the data show that, for the maintained temperatures studied, (between 18° and 30° C.), this minimum is greater for higher temperatures and smaller for lower ones.

CANNON'S results suggest that the relation between root growth and oxygen supply to the roots may be generally found to be markedly influenced by the temperature of the soil (*i. e.,* of the roots) and also that the growth-temperature relations may prove to be correspondingly influenced by the oxygen-supplying power of the soil. Of course, it must be remembered that these relations are both surely influenced by many other kinds of conditions as well.

In his later publications CANNON (**6, 7**) has presented some evidence to the effect that the oxygen supply to roots of plants with aerial parts may, in some cases at least and to a considerable degree, depend upon downward movement of oxygen through the plant itself, from its aerial portions to the subterranean parts. It seems that this downward movement, which would constitute an *internal* oxygen supply to the roots, occurs much more rapidly during periods of photosynthetic activity in the leaves than in periods when the foliage is in darkness, and that it may sometimes be so rapid in periods of illumination of the leaves that oxygen may actually be given off from roots into the surrounding soil. If CANNON'S as yet confessedly meager evidence is reliable, and there is every reason to suppose that it is, his suggestion that roots may in many instances secure an important supply of oxygen by the internal route, is an

exceedingly pregnant and important one for plant physiology, ecology, horticulture, agriculture and forestry. The present writer is not aware that this suggestion has ever before been given prominence. If correct, its importance can not be over-emphasized and it demands prompt and thorough investigation. Following this very valuable lead of CANNON's, it may be supposed: (1) that, in some or many instances, a greater or smaller part of the oxygen used by growing roots reaches them through the plant body itself, moving downward from the leaves, thus not being dependent directly upon the oxygen-supplying power of the root environment; (2) that another larger or smaller portion of the oxygen used by roots is absorbed from the soil, as in the case of the germinating seeds of the present studies; the magnitude of this portion being presumably dependent in general on the oxygen-supplying power of the soil; (3) that some of the oxygen absorbed from the soil by roots may have arrived there by previous outward movement from the same or other roots, especially during periods of foliar photosynthesis, with high partial pressure of oxygen in the illuminated green tissues; (4) that some of the oxygen moving downward through the plant may initially have entered the aerial parts from the air, via stomata, lenticels, etc.; and (5) that some of this descending oxygen may have been formed *de novo* by photosynthesis in the green leaves and, as molecular oxygen, may never have entered the plant at all. The last suggestion may be considered in connection with the downward movement of such substances as dextrose which has long been certainly known to move from green leaves to roots, though by just what means still remains uncertain. It may be that photosynthesis should receive emphasis as an oxygen-producing process occurring within the plant body, as well as being considered as a carbohydrate-producing process. And it may be that the mechanism of the descent of dextrose is the same or is related to the mechanism of the descent of oxygen.

The recent publications by CANNON, referred to in the preceding paragraph, present a large body of experimental data on the oxygen relations of roots, secured by new and ingenious methods, as well as much valuable discussion. They need not be further reviewed here, but readers interested in soil aeration in relation to root growth should refer to them. The results of CANNON's long-continued studies in this field seem to be generally consistent with those of the present experiments on seed germination described earlier in this paper, and it seems probable that the general principles of the oxygen relations for seed germination may generally or in many cases run parallel with those for the maintenance of health and growth in the roots of more mature plants, CANNON's suggestions about internally supplied oxygen being of course borne in mind. If it be assumed that the oxygen-supplying powers in CANNON's root environment

were approximately measured by the percentage of oxygen (partial pressure) in the soil atmosphere, it becomes possible to make a first and tentative comparison between that writer's results on the growth of maize roots and the results of the present studies on the germination of maize seed. With a temperature of 18° C. (not greatly different from that used in the germination experiments of the present studies) CANNON found that the minimum oxygen content (in the gas phase of the soil) that would allow good growth in maize roots was 10 per cent. Correspondingly, the minimum oxygen requirement for the germination of maize seed was found in the present studies to be between 3.0 and 2.3 mg. per square meter per hour. It is at any rate possible to state from CANNON'S results that, with a temperature of about 18° C., the oxygen-supplying power necessary for good growth of his maize roots was presumably about five times as great as the corresponding requirement for his *Potentilla* roots. In the present studies seed-germination tests were not made with any seed having an oxygen requirement between that of maize and rice, but it seems probable that CANNON'S *Potentilla* roots had an oxygen-supplying-power requirement more like that of germinating rice seed than like that of germinating maize seed. It will be remembered that rice seed germinates with an exceedingly low supply of oxygen.

It is perhaps unfortunate that the concept of the oxygen-supplying power of the soil has not previously attracted the attention that it clearly deserves and that earlier studies of soil aeration and of the relations of roots to soil oxygen have necessarily been based on considerations of the static partial pressure of oxygen in the gas phase of the soil. The relations between the percentage oxygen content of the soil air and the oxygen-supplying power of the soil need to be experimentally determined for a series of typical soils and for different water contents before quantitative comparisons may be attempted between the two kinds of measurements. It may be hoped that these relations will soon be studied by investigators in this very important province of physiological ecology. If it be true that the partial pressure of oxygen in the soil atmosphere may safely be considered as proportional to the oxygen supplying power of the soil, then that supposition surely requires experimental support. It seems likely, however, that these two soil characteristics may not be found to be generally proportional though they may well be so in such experimental procedures as those of CANNON. It should be emphasized that the carbon-dioxide-supplying power of the soil also greatly needs attention. It is desirable that the point of view and the method presented in the present paper be modified or replaced as far as logical and practical needs may be brought to light by further experience, and that natural and field soils be studied with respect to their oxygen-supplying powers and their carbon-

dioxide-supplying powers. It is also very desirable that experimental studies be undertaken with controlled conditions as well as for natural and field soils.

V. Summary and conclusions

The studies reported in the present paper have resulted in making available an exceedingly sensitive, practical method for comparing the oxygen-supplying powers of different environments. The method is based essentially on a colorimetric determination of the time required to produce a standard color change in an alkaline aqueous solution of pyrogallol, by the absorption of the oxygen brought to the indicator in a continuous stream of gas, this oxygen having been absorbed from the environment in question by a very thin-walled, water-impregnated, porous porcelain conical-shaped absorber that has capacity for absorbing oxygen and delivering it to the gas stream at least as rapidly as that element is supplied to the absorbing surface by the surroundings. The numerical results, called here indices of the environmental oxygen-supplying power, are expressed as milligrams of oxygen as supplied per hour through a square meter of absorbing area exposed to the environment in question.

During the development of the method a number of comparisons have been made with absorbers placed at different depths in soils of different moisture contents and degrees of packing. The results seem to establish clearly what has generally, but without direct evidence, been supposed to be true, namely, that the oxygen-supplying power of the soil environment is greater near the soil surface and for dryer and more loosely packed soils, while it is less at greater depths and for wetter and more firmly packed soils. With very wet, loamy garden soil the index value for a depth of 40 cm. may be only 1/140 (about 0.7 per cent.) as great as the corresponding value for a depth of 15 cm. Of course, for the same depth, moisture content, etc., the oxygen-supplying power may be expected to vary also with the kind of soil, whether sandy, loamy, clayey, etc., as well as with the prevalence of aerobic organisms and readily oxidizable materials in the soil between the absorber and the air-soil surface. Doubtless soil temperature also will be found to be influential, and barometric pressure and other climatic conditions may not be without influence. With wetting and partial drying of the soil, the index value for a given depth of a given soil fluctuates markedly, being generally smaller when the soil above the depth in question is wetter, and greater when it is dryer. It may be possible to classify field soils with regard to the relations obtaining between their oxygen-supplying powers and the climatic and crop conditions to which they are subject.

The apparatus and method devised for this work appear to be adequate for the experimental study of the dynamics of the soil-oxygen aspects of

ecology and agriculture. They should be valuable in many lines of research dealing with soil organisms as well as with plant roots. Also, the general principles involved should be valuable in studies of other environments besides soil environments, for other processes than vital ones and for some supplying powers other than that for elementary oxygen. Doubtless the method and apparatus may be improved in many ways, but the general principles involved seem to be clear. The definite comparisons between different environments, with respect to oxygen-supplying power, that now become possible should throw light on many problems in physiology, pathology, bacteriology, ecology, horticulture, agronomy, forestry, entomology, etc.

Some preliminary tests were made to gain an idea of the oxygen-supplying power requisite in an environment for the germination of several different kinds of seeds. Wheat germinates well, when well supplied with water and with temperatures about 22° C., in a soil with an oxygen-supplying power of 3.0 mg. or more per square meter per hour; while rice seed germinates well when the corresponding index value is as low as or lower than 0.5 mg. Wheat apparently failed to germinate at all with oxygen-supplying power rates below 1.5 mg. Rice germinated with the lowest supplying-power values tested in these studies. Each kind of seed appears to have its own minimum oxygen requirement but further studies will be necessary before seeds may be definitely classified on this basis.

These results regarding the oxygen-supplying-power requirement for seed germination are in accord with what might have been expected, that this requirement is not the same for all kinds of seeds, some seeds germinating well with exceedingly slow oxygen supply while others require a very rapid supply of that substance. The dynamic point of view and the quantitative method now introduced into the consideration of the oxygen need of germinating seeds may be valuable in the further advance of the physiology of seed germination and also in connection with germination problems encountered in ecology, agriculture, horticulture, forestry and related fields.

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Washington, D. C.

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NUTRITIONAL STUDIES ON *FUSARIUM LINI*[1]

ERNEST SHAW REYNOLDS

(WITH THREE FIGURES)

I. Preliminary studies of some sugars and some nitrogen sources

The study of the nutritional relationships of parasitic fungi is fundamental to a satisfactory knowledge of the diseases which they produce as well as to a correct understanding of the causes of infection, parasitic invasion and resistance. The problem of resistance to disease is undoubtedly very complex, since not only are there many types of parasitic invasion, but there are also many contributing causes which interact with parasitic invasion to give rise to a given diseased condition. The nutritional demands of the invading organism must, however, be met by the host in order to have a successful invasion. Therefore a knowledge of the demands made by specific fungi, and of the substances available in their hosts, will lead to a better understanding of the conditions which make disease possible in those specific cases. Hence it is necessary to know the range of food substances of a given parasite and those available to it in a given host. Resistance as a *nutritional* problem, then, may be due either to the presence of antagonistic substances, or to the absence of essential food requirements, or to various combinations of these. The direct object of the present research is to learn the nutritional requirements and range of specific parasitic organisms, and, in specific hosts, the type, range and abundance of materials which may affect the growth of the parasite. The ultimate object is to give a more definite idea of the causes of resistance, especially where resistant and susceptible strains of the same host are known. It may well be expected also that careful attention to this subject will reveal many facts which will bring about a better understanding of the physiology of the higher plants. The physiological investigation of *obligate* parasites is difficult and the methods of attack upon the problems involved are vague and uncertain. It seems more hopeful to begin the study with organisms which are on the border line between saprophytism and parasitism and to progress toward the obligate parasites from that direction, building upon the knowledge gained from research on the facultative organisms. Such a program has been adopted in the physiological work of this department. A general survey of

[1] This is one of a series of investigations being conducted by the writer and his graduate students. The data upon which this paper is based were largely obtained by Mr. GEORGE C. MAYOUE, under the direction of the writer.

investigations upon fungous nutrition shows that relatively few intensive studies of a single parasitic organism have been made. The accumulation of information upon this subject is largely from rather scattered and more or less unrelated studies upon a number of organisms. It has seemed best, therefore, to attempt a rather complete investigation of one organism in order to correlate the various physiological facts with one another. In seeking an organism which would be appropriate for a continuous study our attention was naturally drawn to *Fusarium lini* Bolley, the causative organism of our common flax wilt disease. Since this fungus can be cultivated readily upon natural and artificial media, is rather widely tolerant of changes in its nutritional sources, and yet is known, up to the present time, only as a parasite upon flax, it seems well suited to the purpose already stated. Another feature which makes it of special interest is that certain strains or varieties of flax are very resistant to its attacks while others easily succumb. Moreover, the fact that it is of wide economic importance certainly does not lessen the advisability of making a careful study of its physiology.

Review of previous work

The pathological examination of the flax wilt disease was largely carried on by Bolley (**1**) but no exact studies upon the nutrition of the fungus seem to have been published. Tisdale (**4**) in 1917 published an account of the inheritance and general nature of the disease in which a few nutritional questions received only incidental attention. The same investigator demonstrated that the flax wilt fungus will scarcely attack the host below 10° C. and thrives best at about 26–28° C. The most direct attack upon the problem of the nutrition of this fungus has been made by Tochinai (**5**). Several special sources of carbon were tried in comparison with one another for a period of two weeks. The amount of fungous growth was determined by weighing the dried mycelium. Representative carbohydrates, organic acids, higher alcohols and phenol derivatives were used as carbon sources. Inorganic and a few complex organic nitrogen compounds and amides were used as nitrogen sources. It was concluded that glucose and the disaccharides, sucrose and maltose, are the best carbon sources, while organic nitrogen was most available to this fungus.

Tochinai further studied various vegetable constituents as the chief source of food, but these gave no data as to the exact requirements of the fungus. He also used a synthetic medium with cane sugar and ammonium nitrate as the sources of carbon and nitrogen. The diversity of cultural conditions that could be used showed, however, the wide range of substances which this fungus may use as its source of nutrition. Conidium and chlamydospore formation varied somewhat with the type of medium employed.

The thick-walled spores were produced especially in those cultures which were unfavorable to mycelial formation.

Preliminary sugar studies

It seemed desirable as a foundation for later work to determine the amount of growth of *Fusarium lini,* which various sugars would produce. Fermi's medium, with the glycerine replaced by the various sugars, was adopted. Its composition is magnesium sulphate 2 grams, monopotassium phosphate 1 gram, dibasic ammonium phospnate 10 grams, and distilled water 1000 cc. The sugars[2] used are classified as follows: The pentose monosaccharides were represented by arabinose and xylose; the hexoses were levulose, d-galactose, glucose, and mannose. The two disaccharides were sucrose and maltose. For most sugars the concentrations used were M/5, M/10, M/15, and M/20 solutions; but M/4 was used additionally for the pentoses, M/40 for the disaccharides and M/25 for glucose.

Cultures were prepared so that each 125 cc. Erlenmeyer flask contained 40 cc. of the medium, and enough flasks were provided so that, for each sugar tested, two could be removed at the end of each week for the duration of the experiment. This means that for each sugar, fourteen flasks were usually prepared, although some sugar cultures were carried for ten or for fourteen weeks. The entire set was inoculated from *Fusarium lini* cultures which had been derived from a single spore isolation, and had also been tested for pathogenicity on flax plants in soil. The effort was made to inoculate the culture with equal quantities of mycelium and spores, although a preliminary experiment has shown that when cultures are carried as long as these the amount of inoculum makes little difference in the final maximum weight. The dry weight of fungus growth was determined for each culture and the results of each pair finally averaged. Check cultures of standard Fermi's medium in which glycerine was present were treated each week similarly to the sugar cultures.

The numerical results are summarized in table I.

It may be seen from these data that the nutritional value of the sugars is in the following descending order: glucose > maltose > sucrose > levulose > xylose > arabinose > mannose > galactose. The M/20 concentration of the disaccharides gave more fungous growth than the corresponding concentration of glucose, while in all higher concentrations glucose surpassed these other two sugars as a source of carbon supply. In the M/20 cultures

[2] The glucose, d-galactose, sucrose and maltose used were Pfanstiehl sugars put out by the Special Chemicals Company. They are the most highly purified sugars obtainable and are essentially free from contaminating sugars or other carbohydrates (2). The xylose, arabinose and mannose were from the Digestive Ferments Company and the levulose from Merck and Co.

TABLE I

DRY WEIGHT OF *F. lini* IN SUGAR CULTURES. AVERAGE DRY WEIGHT GIVEN IN GRAMS

CONCENTRATION OF SUGAR	WEEK	ARABINOSE	XYLOSE	GLUCOSE	MANNOSE	GALACTOSE	LEVULOSE	SUCROSE	MALTOSE	CHECK CULTURE
M/4	1	0.170	0.176	.....	.....	.....	.....	.....	.....	.....
	2	0.187	0.198	.....	.....	.....	.....	.....	.....	.....
	3	0.240	0.239	.....	.....	.....	.....	.....	.....	.....
	4	0.258	0.272	.....	.....	.....	.....	.....	.....	.....
	5	0.263	0.276	.....	.....	.....	.....	.....	.....	.....
	6	0.262	0.270	.....	.....	.....	.....	.....	.....	.....
	7	0.261	0.272	.....	.....	.....	.....	.....	.....	.....
M/5	1	0.074	0.098	0.183	0.183	0.170	0.169	0.123	0.169	0.0105
	2	0.104	0.169	0.295	0.194	0.176	0.187	0.187	0.194	0.0224
	3	0.211	0.219	0.379	0.209	0.206	0.229	0.286	0.300	0.0297
	4	0.198	0.241	0.404	0.234	0.219	0.282	0.305	0.366	0.0324
	5	0.188	0.269	0.433	0.226	0.215	0.294	0.319	0.390	0.0361
	6	0.172	0.262	0.464	0.215	0.216	0.291	0.316	0.400	0.0417
	7	0.170	0.214	0.473	0.220	0.216	0.285	0.312	0.404	.....
M/10	1	0.062	0.070	0.098	0.085	0.079	0.074	0.118	0.141	.....
	2	0.105	0.114	0.194	0.102	0.109	0.108	0.145	0.171	.....
	3	0.163	0.179	0.324	0.201	0.187	0.191	0.282	0.280	.....
	4	0.122	0.180	0.389	0.215	0.208	0.250	0.305	0.323	.....
	5	0.108	0.124	0.343	0.210	0.216	0.251	0.346	0.364	.....
	6	0.095	0.122	0.331	0.213	0.221	0.245	0.359	0.391	.....
	7	0.094	0.102	0.328	0.211	0.215	0.235	0.358	0.391	.....
M/15	1	.....	.....	0.088	0.062	0.049	0.040	0.024	.....	.....
	2	.....	.....	0.175	0.082	0.101	0.094	0.091	.....	.....
	3	.....	.....	0.213	0.141	0.186	0.169	0.181	.....	.....
	4	.....	.....	0.284	0.187	0.198	0.194	0.198	.....	.....
	5	.....	.....	0.298	0.192	0.197	0.197	0.208	.....	.....
	6	.....	.....	0.290	0.188	0.194	0.196	0.210	.....	.....
	7	.....	.....	0.281	0.181	0.198	0.192	0.215	.....	.....
M/20	1	.....	.....	0.027	0.008	0.0095	0.009	0.073	0.092	.....
	2	.....	.....	0.092	0.010	0.0109	0.0118	0.114	0.134	.....
	3	.....	.....	0.122	0.015	0.0165	0.0190	0.177	0.198	.....
	4	.....	.....	0.129	0.0135	0.0131	0.0154	0.203	0.219	.....
	5	.....	.....	0.084	0.013	0.0131	0.0138	0.214	0.216	.....
	6	.....	.....	0.071	0.0125	0.0125	0.0136	0.218	0.219	.....
	7	.....	.....	0.062	0.0116	0.0121	0.0134	0.215	0.222	.....

the maltose and sucrose are essentially of the same value as the M/10 cultures of the monosaccharides mannose and galactose. This seems to indicate that in these dilute concentrations of the disaccharides, the carbon is as available to the fungus as it is in mannose and galactose, since the carbon in the M/10 monosaccharides is quantitatively about the same as that in the M/20 disaccharide media. In more concentrated solutions the disaccharides are somewhat better carbon sources than these two hexoses. Maltose almost uniformly gave a better growth than sucrose, possibly due to the fact that when it breaks down it gives rise to two glucose molecules while the hydrolysis of sucrose gives one of glucose and one of levulose. The latter is a less valuable carbon source than the glucose. The carbon value of the pentoses relative to the other sugars must be determined in the light of the fact that they contain one less carbon atom per molecule than the hexoses. A culture containing M/4 concentration of a pentose contains somewhat more carbon than a solution of M/5 hexose. In cultures of M/4 concentration using arabinose and xylose, there was somewhat greater growth than in the M/5 galactose and mannose. It would seem therefore that these four sugars are about equal as carbon sources for this fungus when equalized as to the amount of carbon present. Possibly the carbon of the pentoses is a little more available than it is in the two hexoses, galactose and mannose. The glycerine of the check cultures gave uniformly poorer growth than all but the lowest concentrations of sugars, showing that at least in this type of medium it is notably inefficient as a carbon source. These results agree very well with the conclusions of TOCHINAI in so far as there is a duplication of work.

It is interesting to note that WEIMER and HARTER (7) obtained only 0.1094 grams of growth of *Fusarium acuminatum* on a 10 per cent. glucose medium in two weeks, while nearly all of these sugars in much weaker solution, about 3.5 per cent., gave a better growth of *Fusarium lini* in the same period.

PRELIMINARY NITROGEN STUDIES

In another series of cultures of *Fusarium lini* carried for two months the ammonium phosphate of Fermi's medium was replaced by various nitrogen sources. Each nitrogen source was so calculated that the same quantity of nitrogen was present in all cultures. Ten culture tubes of each medium were prepared and the combined dry weight of growth was determined at the end of two months.

All cultures were inoculated with the same strain of fungus. After two months the growth was washed and dried on an oven-dried and weighed filter paper. The net dry weight of each culture is given in grams in table II.

TABLE II

GROWTH OF *Fusarium lini* WITH VARIOUS SOURCES OF NITROGEN

NITROGEN SOURCE	DRY WEIGHT IN GRAMS
Potassium nitrate	1.5133
Calcium nitrate	1.1551
Ammonium lactate	1.1273
Ammonium phosphate, dibasic	1.0233
Asparagin	0.8711
Potassium nitrite	0.6649
Ammonium sulphate	0.3933
Urea	0.2189
Ammonium lactate without glycerine	0.1267
Calcium nitrite	none

From the data it is seen that potassium nitrate, calcium nitrate, ammonium lactate and ammonium phosphate provide the best sources of nitrogen for this fungus under the conditions of the experiment, and in the order named. Asparagin, potassium nitrite, ammonium sulphate and urea are next best in the order given, while calcium nitrite gave no growth. Ammonium lactate did not seem to supply the amount of carbon necessary for satisfactory growth when it was the sole source of both nitrogen and carbon.

TOCHINAI used the same *percentage* of all nitrogen sources in his cultures, thus supplying the fungus with different amounts of nitrogen in the different cultures. In the series here reported the various nitrogen compounds were calculated to supply the same amount of nitrogen in each culture. The results show that, contrary to the conclusions of TOCHINAI, the best sources of nitrogen are not organic materials, but the nitrates of potassium and calcium, with the ammonium salts of lactic and phosphoric acids rather close seconds. This series did not include the indefinite material, peptone, which TOCHINAI included and which in the concentration used was the best source of nitrogen. Both investigations demonstrated that nitrites are poor sources of nitrogen for this fungus.

A culture medium supplying its carbon in the form of glucose and its nitrogen in the form of potassium nitrate would seem to be the best synthetic type for *Fusarium lini* so far discovered.

II. Effects of flax extracts on *Fusarium lini*

As a contribution to the solution of the problem of the cause of resistance of certain strains of flax to the wilt disease, a series of experiments was planned to determine what effects extracts of flax plants would have upon the growth of *Fusarium lini* in pure culture.

The flax plants used were of two strains, one which will be called "susceptible" easily succumbs to the wilt disease in "wilt sick soil" while the second, called "resistant," was of the strain know as NDR No. 114, one of the most resistant types developed by Professor H. L. BOLLEY. The plants were gathered when about eight inches tall, dried rapidly on racks

TABLE III

GROWTH OF *Fusarium lini* IN FERMI'S MEDIUM IN PRESENCE OF EXTRACTS OF RESISTANT AND SUSCEPTIBLE FLAX. DRY WEIGHT OF MYCELIUM IN GRAMS

TREATMENT OF MEDIUM	TIME IN WEEKS	PERCENTAGE AND KIND OF FLAX EXTRACT USED								
		Check	Two per cent.		Four per cent.		Six per cent.		Ten per cent.	
			R*	S*	R	S	R	S	R	S
Series A										
Glycerine not autoclaved, extract autoclaved.	1	0.0110	0.0143	0.0146	0.0210	0.0224	0.0217	0.0229	0.0343	0.0346
	2	0.0219	0.0214	0.0215	0.0269	0.0297	0.0289	0.0291	0.0365	0.0359
	3	0.0307	0.0312	0.0321	0.0333	0.0347	0.0328	0.0359	0.0391	0.0466
	4	0.0319	0.0343	0.0350	0.0349	0.0390	0.0385	0.0403	0.0402	0.0474
	5	0.0360	0.0389	0.0393	0.0398	0.0420	0.0396	0.0434	0.0409	0.0479
	6	0.0401	0.0374	0.0395	0.0393	0.0431	0.0394	0.0450	0.0424	0.0490
Series B										
Glycerine not autoclaved, extract through filter cylinder.	1	0.0110	0.0094	0.0101	0.0031	0.0085	0.0080	0.0091	0.0028	0.0036
	2	0.0219	0.0173	0.0191	0.0050	0.0094	0.0090	0.0111	0.0048	0.0081
	3	0.0307	0.0229	0.0246	0.0096	0.0097	0.0109	0.0131	0.0075	0.0148
	4	0.0319	0.0294	0.0293	0.0130	0.0147	0.0093	0.0139	0.0060	0.0159
	5	0.0360	0.0309	0.0324	0.0196	0.0208	0.0078	0.0136	0.0055	0.0152
	6	0.0401	0.0319	0.0336	0.0200	0.0218	0.0068	0.0130	0.0052	0.0141
Series C										
Glycerine autoclaved, extract autoclaved.	1	0.0106	0.0136	0.0133	0.0204	0.0191	0.0209	0.0216	0.0332	0.0347
	2	0.0209	0.0214	0.0211	0.0266	0.0266	0.0284	0.0289	0.0345	0.0353
	3	0.0294	0.0313	0.0311	0.0334	0.0340	0.0329	0.0371	0.0381	0.0390
	4	0.0319	0.0340	0.0398	0.0337	0.0400	0.0383	0.0392	0.0390	0.0401
	5	0.0363	0.0384	0.0485	0.0392	0.0404	0.0393	0.0396	0.0399	0.0418
	6	0.0416	0.0389	0.0498	0.0384	0.0414	0.0388	0.0402	0.0389	0.0430
Series D										
Glycerine autoclaved, extract through filter cylinder.	1	0.0106	0.0090	0.0096	0.0030	0.0042	0.0075	0.0085	0.0024	0.0036
	2	0.0209	0.0170	0.0174	0.0043	0.0072	0.0048	0.0087	0.0041	0.0050
	3	0.0294	0.0213	0.0224	0.0084	0.0099	0.0078	0.0198	0.0057	0.0069
	4	0.0319	0.0286	0.0305	0.0126	0.0138	0.0092	0.0209	0.0050	0.0072
	5	0.0363	0.0295	0.0318	0.0184	0.0190	0.0063	0.0185	0.0051	0.0077
	6	0.0416	0.0287	0.0329	0.0193	0.0202	0.0054	0.0180	0.0049	0.0066

* R = resistant; S = susceptible.

during the summer of 1922, and stored in boxes until used. They were then ground in a mill and the powder was steeped in water about 48 hours. The liquid was filtered off through glass wool and then through filter paper. This filtrate was sterilized by passing it through a Mandler diatomaceous filter into sterile flasks in which it was stored until needed. Measured amounts of the sterile flax extract were added to Fermi's solution, in such proportions that 40 cc. of medium had the same concentration as the standard medium, and the percentage of flax extract varied as indicated in table III, 2, 4, 6 and 10 per cent. being used. All cultures were inoculated with essentially the same amount of *Fusarium lini*. Two cultures were removed from each set at the end of each week of growth for six weeks, and the entire contents of each was placed on an oven-dried, weighed filter paper and washed with 100 cc. of water. The fungous growth was then oven-dried at 85–90° C. on the filter paper in a weighing glass. The net weights of fungous growth are used as an indication of the amount of growth, under the stated conditions.

Four series of cultures with resistant flax extract were run, two with the glycerine of the medium added before autoclaving, and two with the glycerine added after autoclaving. Four corresponding series with susceptible flax extract were run.

Effects of autoclaving flax extract versus sterilization by diatomaceous filter

In series A and C (table III) the flax extract was autoclaved. It will be seen from fig. 1 that in all the cultures in which autoclaving of the extract occurred there was an evident increase in growth over the check cultures which received no flax extract. It is clear also that the higher percentages, autoclaved, consistently provided a better medium throughout the whole period of growth than either the check or the lower percentages. It will be seen, however, that in a considerable number of cases there was actually a smaller weight of growth at the end of the sixth week than at the end of the fifth (figs. 1, 2 and 3). This did not occur in the check cultures, and in fact no decrease was recorded in them until the tenth week.

In series B and D, table III, the flax extract was sterilized through a filter cylinder and in general the opposite growth relations existed. The ten per cent. extract proved to be a poorer medium than any of the lower concentrations, as shown in fig. 2. All of the various concentrations supported less growth than the check cultures for the corresponding growth periods.

From these observations it is evident that the process of autoclaving caused some profound modification of the flax extract which changed it from a depressor of growth for *Fusarium lini* to an accelerator of its growth.

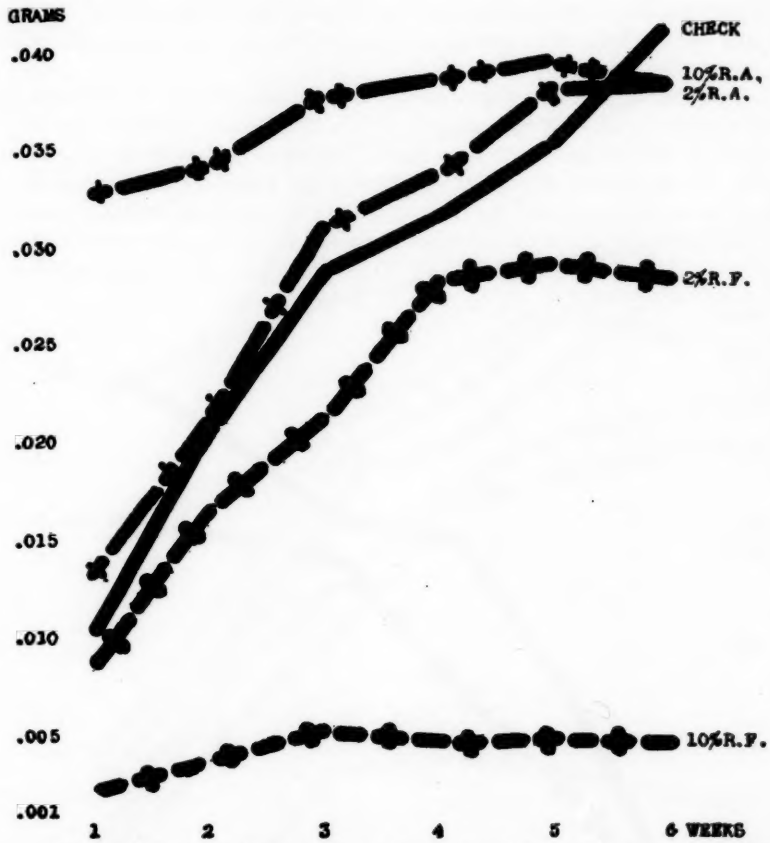

FIG. 1. Dry weight growth curves of *F. lini*. Medium with 2 or 10 per cent. resistant-flax extract (R). Extract autoclaved (A), or filter sterilized and unheated (F). Control culture (check), without flax extract.

This could be caused by several possible changes, such as the destruction of an injurious material, or of an enzyme which was in some manner effective in depressing growth. Or, substances of doubtful food value might be changed to some more available form. That the natural flax extract contains injurious materials is evident from the reduced growth of all cultures on the filter-sterilized extract in series B and D. These are shown, as compared with the check cultures, and with those on the autoclaved extract in fig. 3. The original medium in each case contained the same amount of

nutritive material as the check, but upon the addition of flax extract further foods were added. This is evident from the fact that the autoclaved media gave greater growth than the check.

It is known that flax plants contain a glucoside which is decomposed by an enzyme, producing hydrocyanic acid. This latter substance has been shown by REYNOLDS (3) to be a strong inhibitor of growth for *Fusarium lini*. In the extract which was sterilized in the autoclave the enzyme must have been destroyed and the hydrocyanic acid itself was probably largely driven off, while any food materials contained in the extract were added to those in Fermi's medium (fig. 1). Thus growth of the fungus would be

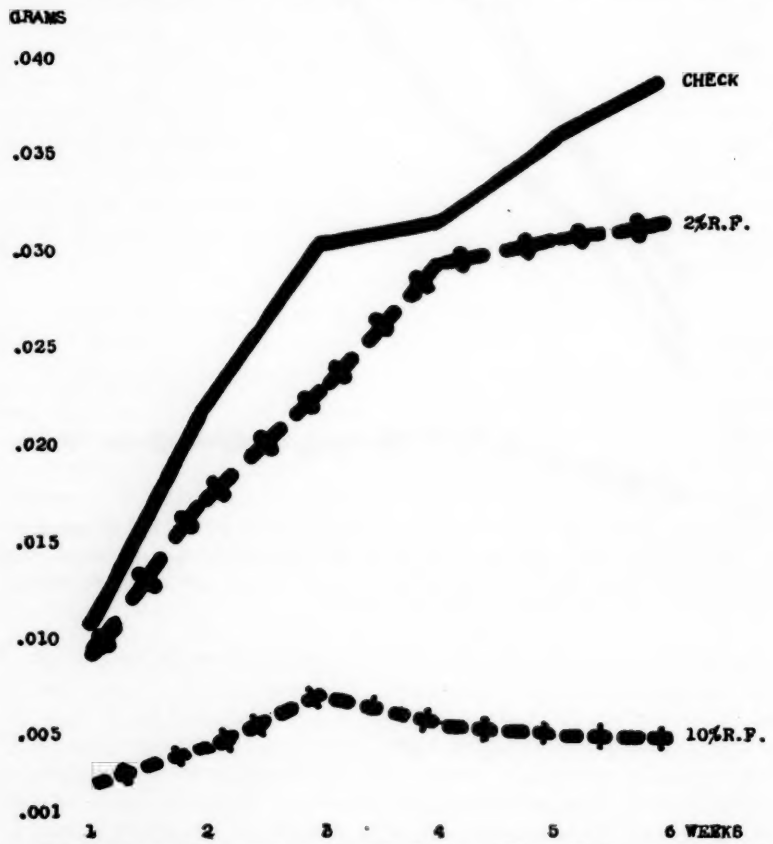

FIG. 2. Dry weight growth curves of *F. lini* on medium with 2 or 10 per cent. of resistant-flax extract (R). Extract filter sterilized and unheated. Control culture (check), without flax extract.

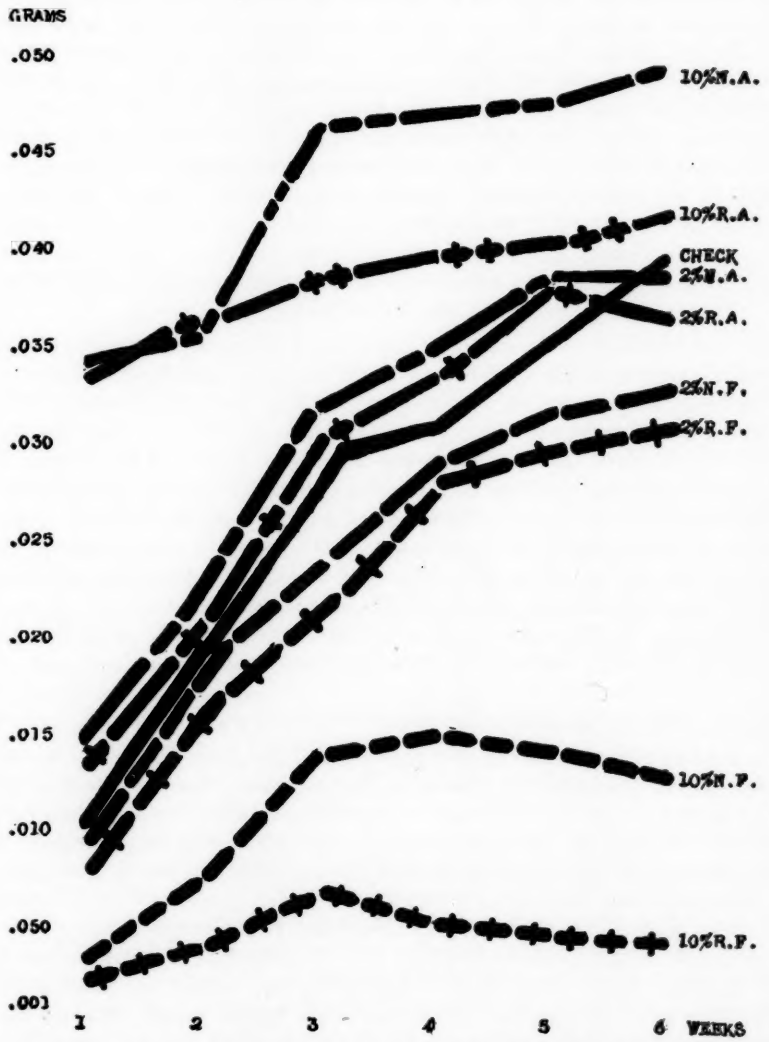

FIG. 3. Dry weight growth curves of *F. lini*. Medium with 2 or 10 per cent. of resistant-flax extract (R), or of nonresistant flax (N). Extract autoclaved (A), or filter sterilized and unheated (F). Control cultures (check), without flax extract.

stimulated in the autoclaved extract roughly in proportion to the amount of extract added. Most, if not all, of the glucoside must have been hydrolized during the time of steeping the flax powder, and an unpublished series of experiments shows that autoclaving may drive off a considerable amount of the hydrocyanic acid in a culture medium. It appears possible, therefore, that the inhibitive substance in the cultures of series B and D was the hydrocyanic acid which was derived from hydrolysis of the glucoside by the enzyme naturally present, while in series A and C the hydrocyanic acid was largely driven off by autoclaving.

Extract from resistant and susceptible flax in relation to growth of *Fusarium lini*

A comparison of the effects of the extracts from the resistant and susceptible strains of flax upon the growth of *Fusarium lini* shows an interesting phenomenon. Almost uniformly the extract from the susceptible flax gave a better growth of fungus than that from the resistant flax (fig. 3). This would show that the factor for depressing growth of the fungus is present in greater quantity in the resistant-flax extract than in that from the susceptible flax. Were the increased growth due only to larger quantities of food materials in the extract from the susceptible strain of flax, we should not find a smaller growth in any case than in the check cultures. However, in all cases in series B and D, the filtered extract, the growth was less than in the corresponding check cultures, though greater with the susceptible than with the resistant extract of equal concentration.

Determination of hydrocyanic acid in flax extracts

As a step in the analysis of the problem of resistance in the flax it was necessary to determine the presence and amount of hydrocyanic acid in the flax extract. The hydrolysis of the glucoside during the 48 hours maceration resulted in the formation of prussic acid, which was demonstrated by a method involving the precipitation of silver cyanide in a standard solution of silver nitrate.

Flax of both the resistant and susceptible varieties was finely ground and equal weights of the powder were steeped for 48 hours in distilled water in Kjeldahl flasks. Then a slow distillation was carried on, passing the distillate into a standardized solution of silver nitrate which converted the hydrocyanic acid contained in the distillate into silver cyanide. After the distillation was fairly advanced phosphoric acid was added and the distillation continued to dryness. One hundred cc. quantities of the distillate were then titrated by a solution of potassium cyanide of known concentration to determine the amount of silver nitrate not combined with the hydrocyanic

acid of the distillate. This titration was carried out in a test-tube. The precipitate produced by adding the potassium cyanide is at first fine, giving a milky appearance to the whole tube. A rather violent agitation, however, flocculates the precipitate and clears the solution so that an end point can be determined by the cessation of the appearance of the milky precipitate. Verification of the end point is attained by filtering and testing one portion of the filtrate with a few drops of silver nitrate and another portion with potassium cyanide. A lack of precipitation in either case verifies the end point.

One hundred cc. of distillate from the resistant flax required 7.8 cc. of the KCN solution to combine with the excess silver nitrate in the distillate. The same quantity of distillate from the non-resistant flax required 9.4 cc. of the KCN solution. Since the same quantity of silver nitrate solution was originally present in the distillate it is evident that the resistant flax extract produced more hydrocyanic acid than the non-resistant flax extract.

Discussion

The four series of cultures reported above, together with other unpublished data, seem to give a reasonable basis for an hypothesis concerning the cause of resistance to the flax wilt disease shown by certain strains of flax. Since the extract from resistant flax, when passed through the filter cylinder, depressed growth of the flax wilt fungus more than the extract from the susceptible variety, it would seem to indicate that the cause of resistance in this strain is a chemical condition. Since the same types of extracts, when autoclaved, accelerated growth, it appears that the factor causing depression of growth is a relatively labile or volatile compound. It is known that in the young flax plant the glucoside linamarin, or phaseolunatin, is present, and that the juices of crushed flax plants change this into glucose and the volatile hydrocyanic acid.

The determination of the relative quantity of prussic acid in the two flax extracts demonstrates that the resistant strain contains more glucoside than the non-resistant. A possible explanation of resistance is that the flax wilt fungus in invading a resistant flax plant with a relatively high quantity of linamarin might easily release sufficient hydrocyanic acid to inhibit its own growth either completely or partially, while in the more susceptible strains there may not be a sufficient production of the poison to prevent the firm establishment of the fungus.[3] Observations have shown that resistance in flax is a relative quality and not absolute, which would be explained by

[3] Since the completion of this work the interesting paper on onion smudge by WALKER (6) and his co-workers has appeared. It is possible that the non-volatile toxic material of colored onions may be related to a cyanogenetic glucoside from which HCN may be released by enzymic action in a manner similar to that suggested for flax wilt.

the facts brought out elsewhere by the writer (3), showing that the fungus is able to grow in cultures that contain the equivalent of at least a .03M concentration of KCN, but is largely prevented from growing in concentrations much higher. Experiments are planned, or under way, to determine the use which *Fusarium lini* may be able to make of various glucosides, the distribution of linamarin in the flax plant, the conditions under which the glucoside is most abundant in the flax, and studies of similar character upon other resistant varieties. It is to be determined also just what effects the flax extracts will have upon the growth of *Fusarium lini* in media which are especially favorable to it. It is expected also to ascertain how other Fusaria and also other parasites of flax are affected by the flax extracts.

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ADSORPTION AS A MEANS OF DETERMINING RELATIVE HARDINESS IN THE APPLE

STUART DUNN AND A. L. BAKKE

Introduction

Hardiness in the apple excites the greatest consideration when one is selecting varieties for an orchard in a northern latitude or in a region with a rigorous climate. The only definite procedure to ascertain this has been to grow the apple tree to bearing age. The underlying reasons why one variety of apple will maintain itself under climatic extremes of drought, heat and cold, while another will not, have been sought since pioneer days. The problem is of particular economic importance in the upper Mississippi valley because there are practically no commercial orchards of winter apples of any kind in this region. Orchardists, nursery men and fruit breeders, unmindful of the size of their task and the time factor involved, have attempted to secure by "trial and error" methods a knowledge of the nature of the conditions producing the qualities they sought. Considering the proposition from any point of view it would seem that a hardy variety must have some constant characteristic or combination of characteristics by which it may be distinguished from varieties which are tender, very early in life, and thus shorten the time and lessen the labor of selection in apple breeding.

In considering a problem of this kind it should be borne in mind that the units of which plant tissue is composed are the cells and it is within these individual cells that the factor or combination of factors which make for hardiness reside. These cellular units may be regarded as colloidal systems, the greater part of which are hydrophilic in nature. It would only be natural to consider that in these colloidal materials are to be found the forces which make for hardiness.

Historical Review

As early as 1860 SACHS (**33**) stated that ice formation in plant tissue takes place principally in the intercellular spaces, and hence rupture of the cells may not be directly attributed to freezing. MÜLLER-THURGAU (**22, 23**) concluded from his experiments that freezing of plant tissue is accompanied by a withdrawal of water from the cell and the formation of ice crystals in the intercellular spaces. On thawing, this water of the inter-

cellular spaces escapes by transpiration, causing the plant to wilt; the killing of the cell may be due to a loss of water. Mez (**19**) on the other hand held that death is due in the case of each particular plant to a fatal "minimum" temperature rather than to injury through direct desiccation.

The results obtained by WIEGAND (**38, 39, 40**) in 1906 are in close agreement with those of MÜLLER-THURGAU. He studied microscopically the effect of low temperature on buds of the horse chestnut and other trees. He was able to detect ice in the intercellular spaces when the temperature had fallen as low as — 18° C. Water probably came out of the cell through the equalizing action of the force of imbibition following the abstraction of water by the ice crystals from the surface film lining the intercellular spaces. He concluded that in no case can the death of any plant be traced directly to absolute cold alone at a temperature below the freezing point. There is a critical point in the moisture content of every cell, beyond which death results due to the actual withdrawal of water to form ice, not to shock, overstimulation, nor any other action of cold. A hardy plant holds water with such tenacity that not enough can be extracted by cold to cause death. Similar conclusions were drawn by WHIPPLE (**37**) in observing winter injury to apple and pear trees in Montana. The work of UPHOF (**36**) on cold resistance in spineless cacti gives results agreeing with those of WIEGAND and MÜLLER-THURGAU.

GORKE (**13**) observed an increase in salt concentration of the cell sap as a result of freezing. He advanced the idea that frost injury was due to the precipitation of proteins through salting out. The effect of the increased concentration of acid salts was considered to be small, however, and insufficient to account for the precipitation. In this statement he was supported by LIDFORSS (**18**), who investigated some of the winter green flora of Sweden. It was found that these plants had in their leaves large amounts of sugar and some oil, but no starch. He proposed the theory that this sugar had a protective action in retarding the salting out of the proteins. BLACKMAN (**5**) pointed out that this theory does not hold in the case of the sugar beet and sugar cane, both of which have a high sugar content but at the same time could not be considered as being in any way resistant to low temperature.

One of the most extensive contributions to this subject is that of CHANDLER (**9**) on the killing of plant tissue by low temperature. Basing his reasoning on the theory of MÜLLER-THURGAU he formulated the idea that some plants might be hardy because the plasma membrane has the property of withstanding great loss of water and that this property might be due, in part at least, to a high sap density or solute content. The force of imbibition which he admits may be the most important is here disregarded. He determined the sap density of a large number of different

plants by using the freezing point depression method. The salt content of the cells was increased by watering the plants with solutions of salts of varying amounts, and distilled water was used on the control plants. His results uniformly showed that the killing temperature was reduced whenever the sap density of the tissue was increased. This apparently shows that the sap density is correlated with hardiness in the succulent plants here studied, but in a later publication (**8**) involving leaves and twigs of apple and peach trees this was not found to hold true. Sap from these tissues was studied to find the relation of its various physical and chemical properties such as freezing point lowering, molecular concentration, and electrolytic conductivity to winter hardiness. No changes, however, could be found that would give any information as to the nature of the change by which plants acquire maturity, or the greater resistance to cold to be observed in winter. Similar conclusions were made by LEWIS and TUTTLE (**17**) who investigated the osmotic properties of leaves of some evergreens at Ontario, Canada. The osmotic pressure of the cell sap was in no case great enough to account for prevention of freezing of tissue.

HARVEY (**14**) made extensive studies on the nature of the hardening process in succulent plants such as cabbage and tomato and found that the principal effect of the hardening process is a change in the constituents of the protoplasm which prevent their precipitation as a result of the physical changes incident upon freezing. The proteins are changed to forms less easily precipitated as indicated by an increase in the amino-acid content of the cabbage plants on hardening. The factors which produce protein precipitation on the freezing of a plant juice are held to be principally the increase in the hydrogen-ion concentration and the increase in the concentration of the salts. The latter factor was held to be insufficient to cause precipitation except under the conditions of a changed acidity. NEWTON (**24**), on the other hand, in an investigation of the hardiness of winter wheat, found no constant relation between the hydrogen-ion concentration, depression of the freezing point, or specific conductivity of the cell sap and relative frost hardiness. He also reported that the relation between water soluble nitrogen and hardiness was not constant.

Working on the question of the dormancy in the plum STRAUSBAUGH (**35**) found that the water in the tissue of hardy trees is held more firmly or in a more stable state than in the tender ones. Microchemical studies showed that dormancy involved a protoplasmic change in the buds that was much more pronounced in the hardy varieties. Protein was evidently modified but just how was not known. He stated that it is possible that there is induced, as a result of these modifications, a very decided change in the colloidal condition within the cells which increases the force of imbibition so that the water of the protoplast is retained against the dehydrating force of freezing.

BEACH and ALLEN (**4**) studied the structure and composition of twigs of numerous varieties of apples to find if these qualities correlate with hardiness. Cutting, compression and penetration tests, as well as specific gravity tests, seemed to indicate some correlation between hardness of wood and hardiness but exceptions were found. Maturity of the wood and low moisture content at the beginning of cold weather is stressed as an important factor. They concluded that from the practical viewpoint it is impossible to name any one test by which the degree of constitutional hardiness of a seedling apple may be foretold. Maturity of tissue as a factor in hardiness was also emphasized by GLADWIN (**12**), SHUTT (**34**), ROBERTS (**29**), POTTER (**28**), CARRICK (**7**), BRADFORD (**6**), HOWARD (**16**), and DORSEY and BUSHNELL (**10**).

The physico-chemical properties of the sap of seventeen apple varieties were tested by BAKKE, RADSPINNER, and MANEY (**3**). Since it appeared that the freezing point lowering difference for different varieties was not in itself sufficient to indicate differences in hardiness these investigators made a series of tests at five different times of the year, not only of the freezing point depression, but of the water content, ash content, and hydrogen-ion concentration as well. The results showed considerable variation, although some correlations to hardiness were found.

HOOKER (**15**) compared hardy and non-hardy apples as to pentosan content of the twigs. Excellent correlation was found between the development of pentosans and resistance to low temperatures. A correlation was also present between pentosan content and water holding and absorbing capacity as measured in a sulfuric-acid-filled desiccator. He advanced the theory that the pentosans, or perhaps some specific pentosan, function in the plant tissue by holding water, which is in the nature of adsorbed or colloidal water, and that this water cannot freeze when the plant is subjected to ordinary winter conditions. ROSA (**30, 31, 32**), working on vegetable plants such as cabbage, cauliflower, lettuce, and tomato, got results similar to those of HOOKER. There was a marked parallel between pentosan content and hardiness, indicating that pentosans may be related in part causally to cold resistance. He concluded that salt content, acidity, hydrogen-ion concentration, sugar, moisture, and protoplasmic colloids other than pentosans are also partly responsible.

Among the more recent contributions on hardiness are two by NEWTON (**24, 25**). Both of them deal with frost resistance in winter wheat. He worked with several varieties of winter wheat which could be divided into three groups: very hardy, medium, and tender. He determined several properties of the hardened leaf tissue to discover any possible correlation. These properties were depression of the freezing point, specific conductivity, hydrogen-ion concentration of the cell sap, dry matter content, water

soluble nitrogen, and sugar content. No constant relation between any of them and hardiness could be found. However, he did find that the imbibition pressure of fresh leaves in the winter hardened condition was in most cases directly related to hardiness. Pressures were as high, in some cases, as 600 atmospheres. The volume of press juice obtained per 100 grams of hardened leaves was inversely proportional to the hardiness of a variety. Unhardened leaves showed neither of these properties. The imbibition pressure of hardened leaves appeared to depend on the physical state of the cell colloids characteristic of living tissues, since this property was lost when the tissue was killed. The quantity of hydrophilic colloids in the press juice of hardened tissues was found to be directly proportional to hardiness.

It is recognized that the colloids found in a growing plant are in competition with the colloids in the soil medium. This is particularly evident in the case of water absorption. To determine the amount of colloidal content present in soils the adsorption of certain dyes from solutions by the soil was found to be a reliable test for colloidal content.

PEARCE and MILLER (**27**) worked on adsorption of dyes by glacial clays. The clays studied were negatively charged in their colloidal form, and therefore should markedly adsorb such positive dyes as methylene blue and should show very little attraction for the negative dye eosin. Such was found to be the case, the adsorption of eosin being relatively slight and therefore somewhat irregular. The dye solutions were shaken with samples of clay weighing one gram or less and then allowed to stand until settled. Equal amounts of the supernatant dye solution were drawn off and the relative color intensity read with a Duboscq colorimeter.

Three different methods were used by ANDERSON, FRY, GILE, MIDDLETON, and ROBINSON (**1**) to estimate the amount of colloidal material in soil samples through adsorption. These were (*a*) water vapor, (*b*) ammonia and (*c*) the dye, malachite green. For this last test a solution of the dye was added to the soil sample (0.25 to 1.0 gram), shaken continuously for one hour, and then centrifuged to throw down the suspended matter. The supernatant liquid was then drawn off and the amount of dye remaining determined by reading with a Duboscq colorimeter against a standard dye solution. It was calculated from the adsorption of mineral powders and the mineralogical composition of soils that as a rule less than five per cent. of the total adsorption of the soil is due to the non-colloidal part. Adsorption by non-colloidal constituents should, therefore, not seriously affect adsorptive methods for determining the amount of colloids in soils. In another publication (**11**) these same workers carried out further tests of colloidality by adsorption of malachite green. The results agreed very closely with tests on adsorption of ammonia gas and of water vapor.

ASHLEY (**2**) in testing clays for plasticity used the dye adsorption method for determining colloidal matter. Brilliant green, malachite green, and gentian violet were some of the dyes used. Malachite green seemed most suitable. He found that the adsorption of a dye by clays supplies an approximate measure of plasticity. MOORE, FRY, and MIDDLETON (**21**) also used malachite green and secured very nearly the same results as when a dry gas is adsorbed by dry colloidal substance. This fact in itself would seem to justify confidence in the results. PATTEN and WAGGAMAN (**26**) used gentian violet quite extensively. This dye was found to be well adapted for studying distribution of solute between soil and solution because it was changed very slightly by contact with soils used and because it can be readily estimated colorimetrically to within a fraction of a part in a million of solution.

WILKINSON and HOFF (**41**) experimented on the adsorptive power of soils using methylene blue, diamine blue 3B, and neutral violet. MILLER (**20**) in studies on adsorption by activated sugar charcoal was able to show that soils and charcoal have in common the property of removing from solution many of the same types of electrolytes such as the basic dyes, malachite green and methylene blue, acids, salts, etc. The dyes seemed to be held in much the same way by these adsorbents.

It appeared that similar tests applied to plant tissue might also indicate its colloidal content and such results might correlate with observed hardiness. It was with this end in view that these investigations were undertaken.

Materials and methods

The material used in this work was taken from twigs of twenty-two different varieties of apples grown in the Scion Orchard of the Mount Arbor Nursery at Shenandoah, Iowa. They were all well known commercial varieties possessing a graduation in resistance to unfavorable weather conditions from extremely hardy down to the rather tender. In general they might be arranged into three groups. Among the more hardy are such varieties as Hibernal, Patten Greening, McIntosh, Oldenberg or Duchess, Wealthy, Virginia Crab, and Red Astrachan. At the other extreme among the markedly tender ones are Ben Davis, Stayman, Winesap, Baldwin, Delicious, Maiden Blush and Winter Banana. Somewhere between these will be grouped those medium in hardiness, such as Malinda, Fameuse, Peerless, Salome, Roman Stem, Northwestern Greening, and Yellow Transparent.

The twigs were all gathered November 11, 1924, from trees that were growing under as nearly the same conditions as possible. Maturity was at hand as the growing season was ended. The twigs were ground rather coarsely in a power food chopper at once, then carefully air dried, after

which they were ground more finely in a disk power mill. The material was sifted through a 100 mesh sieve. Nearly all that would pass through was found to be composed largely of cambium, cortical and other non-woody tissue. That remaining was woody tissue very easily distinguished because of its fibrous nature. For purposes of brevity, the material which sifted through will be here spoken of as cortical and the other as non-cortical. Since the region thus designated as cortical is the seat of most of the growth of the twig it may thus be regarded as the most vital part. It was thought to be the logical tissue on which to make tests for ability to withstand winter killing. These investigations were therefore centered on this tissue.

In these tests such dyes as methylene blue, acid fuchsin, eosin, gentian violet, and malachite green were used. Stock solutions of the dyes were made up at a strength of 0.1 gram of dye to 100 cc. distilled water and these were diluted in varying amounts for different tests. For the tests with eosin the stock solution was diluted 1 to 10. A sample of tissue weighing 0.5 gram was placed in a test bottle and to it was added 25 cc. of dilute dye solution. It was shaken at frequent intervals for one hour and then placed in a centrifuge and whirled for five minutes at a speed of 1,000 revolutions per minute. The bottle was then removed and the clear supernatant liquid decanted into one of the cups of a Duboscq colorimeter. In the other cup was placed some of the pure dye solution and the amount of dye removed by the sample found by comparison.

Results

In the case of eosin several samples indicated no adsorption by the material. Acid fuchsin also gave no adsorption indicating that the material is negatively charged like the soils studied by PEARCE and MILLER (**27**). Consequently no further tests were made with these dyes.

In working with methylene blue, several dilutions of the stock solution were tried and the one most practicable was found to be 1 to 32, that is, a match of colors in the colorimeter was most easily obtained at this dilution. This dye showed a very marked adsorption by all of the samples. Since these tests are only comparative, one of the most convenient ways of expressing the results is by the fraction of dye left in solution after treating the sample, which is obtained by dividing the reading of the standard by the reading of the solution tested. The results of this series of tests appear in table I arranged in the order of their numerical magnitude.

By this arrangement there are five varieties that do not conform to the general arrangement of approximate hardiness. These are Northwestern Greening, Hibernal, Wealthy, Oldenberg, and Patten Greening. The rest, although not grouped exactly in the order of known hardiness, yet arrange themselves in three general groups, hardy, semi-hardy, and tender.

TABLE I

ADSORPTION OF METHYLENE BLUE BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Red Astrachan	37.0	0.7	.019
McIntosh	27.2	0.6	.022
Northwestern Greening	29.1	0.8	.027
Virginia Crab	25.2	0.7	.029
Peerless	17.8	0.5	.028
Salome	22.9	0.8	.035
Yellow Transparent	28.5	1.0	.035
Jonathan	21.2	0.8	.038
Malinda	25.4	1.0	.039
Stayman	33.3	1.4	.042
Roman Stem	26.2	1.2	.046
Hibernal	23.7	1.1	.046
Wealthy	29.1	1.4	.048
Winesap	13.6	0.7	.051
Winter Banana	27.1	1.4	.052
Oldenberg	21.7	1.4	.064
Ben Davis	20.6	1.4	.068
Patten Greening	14.6	1.0	.068
Delicious	11.2	1.0	.089
Fameuse	22.0	1.6	.092
Maiden Blush	45.1	4.4	.098
Baldwin	33.3	3.9	.117

Gentian violet was then tried in varying dilutions and with varying hydration periods. In some series of tests the samples were allowed to stand as long as over night in the dye solution in order to determine whether the time of hydration might be a factor. No better correlation to hardiness than the results given in the first table was found.

Lastly, malachite green was used. As with the other dyes a stock solution was made up at the rate of 0.1 gram of dye to 100 cc. distilled water. Samples of material weighing 0.5 gram were likewise used. Several series of tests with varying dilutions of the stock solution and varying hydration periods from one hour to several hours were made but in no case was the correlation any better than before.

With the previous tests the material was allowed to remain in the dye solution for a considerable length of time, usually at least an hour and in some series of tests as long as over night. It might be supposed, then, that this length of time might allow considerable alteration both of the dye itself and of the colloidal matter. Acting on this hypothesis in an effort to secure

a better correlation a series of tests was made in which the time of treatment was greatly shortened. The stock solution of malachite green was diluted 1 to 15 for this series. The half gram sample and 25 cc. of the dye solution were shaken for 15 seconds and centrifuged for 10 minutes after which comparison was at once made in the colorimeter. The results of this short time test appear in table II.

TABLE II

ADSORPTION OF MALACHITE GREEN BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Peerless	43.2	2.0	.0463
Wealthy	42.0	2.0	.0577
Hibernal	46.6	2.3	.0501
Red Astrachan	42.8	2.3	.0537
Virginia Crab	23.5	1.3	.0554
McIntosh	44.5	2.5	.0562
Oldenberg	42.0	2.4	.0572
Maiden Blush	36.8	2.3	.0625
Patten Greening	41.5	2.9	.0652
Yellow Transparent	44.5	3.0	.0675
Malinda	40.0	3.0	.0750
Salome	40.0	3.0	.0750
Roman Stem	39.5	3.0	.0760
Stayman	35.0	2.7	.0771
Winter Banana	41.2	3.2	.0777
Fameuse	42.0	3.4	.0809
Jonathan	35.6	3.0	.0843
Northwestern Greening	43.7	3.2	.0951
Baldwin	41.0	5.0	.1220
Winesap	40.0	5.0	.1250
Delicious	40.0	5.1	.1270
Ben Davis	40.0	5.5	.1380

Here it will be noticed that the correlation to hardiness is much better. Only about three or four varieties are noticeably placed differently from the hardiness grouping, notably the Maiden Blush, Stayman and Fameuse. Fameuse is usually regarded as rather hardy and Stayman and Maiden Blush as tender.

Early in these investigations it was found difficult to obtain a good match of the colors when comparing them in the colorimeter due to coloring matter dissolved from the tissue itself and which usually imparted a brownish or yellowish tinge to the solution. An effort was made to counteract

this by use of amber glass color screens but this was not entirely satisfactory. Finally the scheme was tried of treating 0.5 gram samples of tissue with distilled water only and using the resulting colored solution as a color screen. This was done for each variety and it was suspended in a vial beneath the cup containing the standard by means of a wire. By using this form of compensator excellent results were obtained. It was possible to obtain a much better match of colors than before. The results of a series of tests with malachite green at a dilution of the stock solution of 1 to 10 appear in table III.

TABLE III

ADSORPTION OF MALACHITE GREEN BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Patten Greening	43.2	1.0	.0232
Yellow Transparent	35.0	0.9	.0257
Oldenberg	35.0	1.0	.0285
Virginia Crab	28.0	0.8	.0285
McIntosh	39.8	1.2	.0302
Hibernal	41.6	1.3	.0312
Peerless	48.0	1.7	.0354
Red Astrachan	47.5	1.7	.0358
Wealthy	37.0	1.4	.0378
Malinda	44.9	1.8	.0401
Northwestern Greening	38.3	1.6	.0417
Salome	42.8	1.9	.0444
Fameuse	43.0	2.0	.0465
Jonathan	34.4	1.6	.0465
Roman Stem	42.0	2.0	.0476
Maiden Blush	37.0	1.8	.0476
Winter Banana	30.0	1.5	.0500
Winesap	41.5	2.1	.0506
Stayman	41.0	2.2	.0536
Baldwin	41.0	2.3	.0561
Delicious	41.0	3.0	.0733
Ben Davis	40.2	3.7	.0920

Here it will be noted that the varieties are grouped very nearly in the order of actual hardiness, indicating that the adsorptive powers of the colloids present are correlated with hardiness. Experienced orchardists, nurserymen, and horticulturists disagree as to the exact relative position of some of these varieties but in general the grouping will be much as given in the above table, the most hardy first.

Discussion and conclusions

It should be pointed out here that there is a remarkable similarity between the adsorptive behavior of this plant tissue, and that exhibited by the soils and charcoal studied by MILLER (**20**) and the soils studied by PEARCE and MILLER (**27**), ASHLEY (**2**), and others. These materials seem to have in common the property of removing from solution many of the same types of electrolytes such as the basic dyes, malachite green and methylene blue, and show no adsorption with acid dyes. It is well known that the amount of dye these materials will remove from solution has been used as a measure of the adsorptive capacity of decolorizing charcoals and of the clay or colloidal constituents of soils. The dyes appear to be held in much the same way by these adsorbents as by the plant tissues here tested. After a dye has been removed from aqueous solution by either of these three substances, it may be recovered unaltered from the adsorption complex, in part at least, by simple extraction with alcohol, acetone, or other organic solvents. It is interesting and perhaps significant that such apparently different substances should have so many of these properties in common. Soils and this pulverized plant tissue are very complex materials which afford possibilities for many types of reactions, while pure charcoal, which is ordinarily considered to be very inert chemically, can give rise to only one type of reaction and that by virtue of the forces existing at its surface.

The results of these tests indicate rather conclusively that the hydrophilic colloids of the plant are in a large measure concerned in hardiness. In this they agree very closely with the results of NEWTON (**25**), HOOKER (**15**), ROSA (**32**), and others, whose work on colloidal matter of plant tissues has shown a close correlation between the quantity of such material present, and hardiness. That is, it appears certain that the hydrophilic colloids by reason of their relatively enormous adsorptive surfaces hold water within the cell and prevent death from the dehydrating force of freezing. Furthermore, these results indicate that this property may be quantitatively measured in a convenient manner by the adsorption of aniline dyes from solution.

In the tests that have been made it is clear that these measurements of adsorption may be conveniently and advantageously used in measuring the relative hardiness of different varieties of the apple.

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A STUDY OF THE CLEARING OF ALCOHOLIC PLANT EXTRACTS*

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Clearing is generally a necessary part of the analytical procedure with plant materials preserved and extracted with 80 per cent. alcohol, as advocated in a previous paper (**3**). Clearing is equally necessary when samples, killed and preserved by drying, are not given a preliminary extraction with ether before extracting with alcohol. The work reported here suggests that this necessity is based more upon the mechanical removal of lipoids, waxes, and certain colloids not precipitated by 80 per cent. alcohol, than upon the chemical removal of copper reducing substances. It is probable that comparable, although somewhat high, reducing-sugar values may be obtained with certain alcoholic plant extracts without either preliminary ether extraction or clearing. On the other hand, the alcoholic extract from fresh mangel leaves so clogs the filter, when evaporated and taken up in water, as to be unfilterable by ordinary means. The same is true of a large number of leaf, green-fruit, and woody-stem extracts.

Correspondence with a number of physiological chemists indicated a lack of uniformity in clearing methods. A number of men were using neutral and basic lead acetate interchangeably although the work of Bryan (**1**) indicates the danger of such a procedure. With these conditions in mind we have attempted to determine the limits of value and safety in the use of neutral- and basic-lead-acetate solutions for clearing plant extracts.

General procedure

Stock extracts of plant tissues, so chosen as to give a considerable range of clearing conditions, were prepared from fresh green material of known moisture content by dropping the sample into enough boiling 95 per cent. alcohol to give a final concentration of 80 per cent. After boiling for 2 or 3 minutes the samples were sealed and set aside 24 hours or longer. The extract was then decanted off, 2 ml. of 80 per cent. alcohol added for each gram of fresh material used, and the extraction repeated. The extracts were combined and preserved in glass containers. Samples of 100 to 300 ml. were taken for clearing and reduction, depending upon the reducing power of the extract. These were placed in pyrex beakers and the alcohol

* The work reported here was done at Cornell University during the tenure of a National Research Council fellowship in the biological sciences.

removed on a boiling water bath. Evaporating 200 ml. of a celery extract to 20 ml. when strong alcohol fumes were still given off gave a value of 28.0 mgs. dextrose per sample. Evaporating to 2 ml. which completely removed the alcohol gave a value of 29.0 mgs. dextrose. Since the difference of 1 mg. is within the error of weighing, the practice of evaporating to 3 to 5 ml. was adopted. One hundred milliliters of distilled water, less the quantity to be added in the lead solution, were added and heated to 80° C. to soften gummy precipitates and insure the solution of all reducing substances. As a further precaution the sides of the beaker were rubbed with a rubber police and insoluble masses were finely divided. After cooling to room temperature the desired quantity of lead solution was added and the samples filtered immediately into 200 ml. beakers to which had been added an estimated excess of potassium-oxalate crystals.[1]

The lead precipitate was allowed to drain on the filter and was washed with cold water until the filtrate no longer gave a precipitate in the oxalate solution below. The presence of an excess of oxalate was assured by testing with a drop of dilute lead-acetate solution. The deleaded solution, which was kept within a volume of 160 ml., was then filtered into a 200 ml. graduated flask, beaker and precipitate carefully washed, and the solution adjusted to 200 ml. at 20° C. in a water bath. Three 50 ml. portions were taken for copper reduction by the QUISUMBING and THOMAS procedure (**6**). Copper was determined by direct weighing of the cuprous-oxide precipitate. Several samples from each lot were retained and the purity of the precipitate determined by the official, sulphuric-nitric-acid, electrolytic method (**5**). Copper was deposited on a revolving platinum grid cathode by a current of 2 amperes for a period of 55 minutes. Calculations are based on the tables by QUISUMBING and THOMAS.

It was found practicable to hold the weights of the three copper precipitates to a maximum variation of 0.0015 gms. on the lighter and 0.0020 gms. on the heavier samples. This limits the error of any figure included in the data to three-tenths of a milligram of dextrose and requires differences of one to two milligrams for significance.

Experimental results

CONDITIONS AFFECTING THE DESTRUCTION OF REDUCING SUBSTANCES BY LEAD COMPOUNDS

When the preliminary experiments indicated considerable loss in the reducing activity of plant extracts treated with excesses of basic lead acetate,

[1] Potassium oxalate was chosen as a deleading reagent on the basis of the work of SAWYER (**7**), EYNON and LANE (**2**), MEAD and HARRIS (**4**) and experimental data to be presented in a later paper. We have found potassium oxalate both safe and convenient when used in excess and the precipitate allowed to crystalize before filtration. The practice of filtering the cleared solution onto the oxalate crystals allows both of these conditions to be met.

it became important to determine the conditions under which these losses could be reduced to a minimum. One milliliter of 1.25 sp. gr. lead-acetate

TABLE I

THE RELATIONS OF TIME AND TEMPERATURE TO THE DESTRUCTION OF REDUCING SUBSTANCES BY LEAD COMPOUNDS

SOLUTIONS CLEARED WITH 5 TIMES THE QUANTITY OF LEAD NECESSARY TO GIVE A SLIGHT EXCESS AND DELEADED WITH POTASSIUM OXALATE AS INDICATED	REDUCING ACTIVITY OF MANGEL-LEAF EXTRACT AS A PERCENTAGE OF CHECK (1 ML. NEUTRAL)	
	Cleared with neutral lead acetate	Cleared with basic acetate
Deleaded at once at room temperature	99.3	86.5
Heated for 15 minutes after deleading	93.9	78.6
Heated for 15 minutes before deleading	71.5	47.5
Left at room temperature for 14 hours before deleading	88.6	77.6

solution was sufficient to give a slight excess with the extract used for this experiment. Five milliliters were accordingly used for the comparisons. The data in table I indicate that increasing the quantity of neutral lead acetate used in clearing to 500 per cent. of the check had no effect on the reducing activity of the extract when the lead was removed at once. Basic lead gave a significant loss under the same conditions. Any standing before deleading, or heating with or without deleading (potassium oxalate does not remove all the lead), lowered the reducing activity, but the percentage reduction was always greater when basic lead was used.

A COMPARISON OF NEUTRAL AND BASIC CLEARING

The reducing values of a number of plant extracts cleared with minimum and excess quantities of neutral- and basic-lead-acetate solutions are compared in table II. The minimum dose of lead was taken as the quantity of 1.25 sp. gr. solution which gave a distinct, white precipitate when a drop of dilute potassium-oxalate solution was added to the extract-lead solution mixture. With the samples used, this varied between 1 and 2 ml. of solution. Five times the minimum dose was taken for the excess treatment. For ready comparison the reducing value of the minimum or low-neutral clearing is given in milligrams dextrose and the other treatments are calculated as a percentage of this value. Percentage differences which may be considered to be significant vary from 4 or 5 per cent. with the small reducing values to 1 or 2 per cent. with the larger. The data given in table

II show that, with the exception of the apple-wood extract, an excess of neutral lead acetate which was five times the quantity required for clearing had no effect upon the reducing power of the solutions. The minimum dose of basic lead acetate lowered the reducing power an average of 3 per cent. and this was increased to 11 per cent. by the excess treatment. In addition the variation between best and poorest recovery was about twelve times greater with the excess-basic than it was with the excess-neutral clearing. While a comparison of the absolute quantities of reducing substances in sweet potato roots and mangel leaves will rarely be required, that method which will most nearly give such values is most likely to give reliable results when comparing sweet potato roots of varying composition.

Although an excess of neutral lead decreased the reducing power of apple-wood extract this reduction was apparently due to an incomplete precipitation of reducing impurities when 2 ml. of lead solution were used. When 4 ml. was taken as the check value, high neutral gave a value of 98.4 per cent. which compares with the values obtained with the other extracts. The apple-wood extracts gave a very heavy precipitate with lead acetate and the first formation of a precipitate with potassium-oxalate solution appar-

TABLE II

A COMPARISON OF THE REDUCING POWER OF EXTRACTS CLEARED WITH NEUTRAL AND BASIC LEAD ACETATE SOLUTIONS

CLEARING TREATMENT	LOW NEUTRAL AS MGS. DEXTROSE, OTHER TREATMENTS AS A PERCENTAGE OF THIS VALUE							
	Mangel leaf extract	Tomato leaf no. 1	Tomato leaf no. 2	Sweet potato roots	Apple wood no. 1	Apple wood no. 2	Celery stalks	Spinach leaves
Low neutral	25.5	26.1	40.2	56.3	43.2	97.6	154.2	17.4
Excess neutral	98.8	99.6	99.8	98.6	94.9	94.0	100.0	.....
Low basic	97.7	98.9	99.3	96.8	96.1	92.7	98.3	.....
Excess basic	79.6	85.4	87.8	96.3	85.9	87.4	93.5	88.5

ently did not indicate sufficient excess of neutral lead to complete the removal of active impurities.

COMPLETENESS OF CLEARING.—When a small but distinct excess of neutral lead acetate was used the color of the solutions so cleared compared favorably with that of the best clearing obtained. Low basic was almost invariably less satisfactory than either of the neutral clearings from the standpoint of color and ease of handling. High basic was normally lightest in color, but showed a distinct tendency to darken if allowed to stand after

deleading. Water-clear solutions were obtained with all of the extracts used, by deleading with hydrogen sulphide after adding an excess of neutral lead acetate. Although hydrogen sulphide left the solutions acid to methyl red while they were boiled to expel the excess of the gas, such a treatment gave very close checks with potassium-oxalate deleading on an unhydrolyzed celery extract which contained approximately twice as much non-reducing as reducing sugars. Neutral-lead-acetate-hydrogen-sulphide clearing is recommended for trial when complete removal of coloring matter is required. It has no advantage when reducing sugars are to be estimated with Fehling's solution, and is less convenient. The precipitation of a large excess of lead with hydrogen sulphide will lower the reducing power of the solution, presumably by co-precipitation of reducing substances.

NEUTRAL AND BASIC CLEARING OF PURE REDUCING-SUGAR SOLUTIONS.—Extensive tests were made of the effect of neutral and basic lead acetate on the reducing power of pure sugar solutions and on the purity of the reduced cuprous oxide. The data given in table III are typical of the results obtained and agree with those presented in table II. The low-neutral clearing with potassium-oxalate deleading, which was used as a standard in this work, is shown to give reliable results on pure sugars. Also, a large excess of neutral lead acetate is much less destructive of reducing sugars than is a corresponding excess of basic acetate. There is less difference in the stability of the two sugars than would have been expected from the work of BRYAN (**1**). The same results were obtained, however, when known quantities of levulose and dextrose were added to plant extracts of known reducing value. When 22 mgs. of dextrose were added to an apple-wood extract, 98.2 per cent. was recovered from the high-neutral and 90.4 per cent. from the high-basic clearing. Of 18 mgs. of

TABLE III

THE EFFECT OF CLEARING ON PURE SUGAR SOLUTIONS

TREATMENT SOLUTIONS CLEARED AND DELEADED AS ABOVE—1 AND 5 ML. OF THE LEAD SOLUTIONS WERE ADDED	REDUCING SUGAR FOUND—CALCULATED AS MGS. AND AS PER CENT. OF CHECKS (UNCLEANED)			
	LEVULOSE		DEXTROSE	
	Mgs. sugar found	Recovery as per cent. of check	Mgs. sugar found	Recovery as per cent. of check
Pure solutions (check)	182.9	.....	132.2	.....
Low-neutral clearing	183.0	100.06	132.1	99.92
High-neutral clearing	182.4	99.73	130.8	98.94
Low-basic clearing	179.3	98.03	131.3	99.32
High-basic clearing	159.5	87.21	122.5	92.66

levulose added to parallel samples, 96.1 per cent. was recovered from the high-neutral and 86.6 per cent. from the high-basic clearing. A third series of extracts receiving the same clearing treatments was used as the basis of comparison.

A STUDY OF THE LOSS OF REDUCING SUBSTANCES IN BASIC CLEARING

The consistently lower reducing value of solutions cleared with basic lead may be assumed to be due to one of the following causes or to a combination of them: (1) The loss may be due to the co-precipitation or reducing substances. (2) It may be due to the formation of an insoluble, sugar-lead compound or to the formation of a slightly soluble compound which is co-precipitated with, or adsorbed by the clearing precipitates. Or (3), it may be due to the removal of organic substances which are precipitated and weighed as cuprous oxide when clearing with neutral lead acetate. The last supposition is not in accord with the uniform losses obtained with plant extracts and pure sugars. The uniformly higher dry weights of the excess-basic-clearing precipitates, approximately three times the weight of the excess-neutral precipitates, lends color to the co-precipitation theory.

RELATION OF QUANTITY OF PRECIPITATE TO LOSS OF REDUCING SUBSTANCES.—If the loss of reducing substances in basic clearing is due to co-precipitation alone, then the loss should be proportional to the volume of precipitate and independent of the precipitating reagent. Apple-wood extract was used for an experiment on this question because it gave the heaviest precipitate of any of the materials available. Four samples containing respectively 200, 100, 50, and 25 ml. of extract were made up to 200 ml. with 80 per cent. alcohol, the alcohol driven off and the residue taken up in water and cleared with 2 ml. of neutral-lead-acetate solution. The 100 and 50 ml. samples were best cleared by this quantity of lead and gave slightly lower results.

TABLE IV

THE EFFECT OF A NEUTRAL-LEAD PRECIPITATE ON THE RECOVERY OF REDUCING SUBSTANCES FROM APPLE-WOOD EXTRACT

QUANTITY OF EXTRACT IN SAMPLE, MILLILITERS	WEIGHT OF LEAD PRECIPITATE		REDUCING SUBSTANCES AS DEXTROSE	
	Mgs. per sample	Gms. per liter extract	Mgs. per sample	Gms. per liter extract
Two hundred	887	4.435	88.0	1.760
One hundred	467	4.670	43.1	1.724
Fifty	243	4.860	21.6	1.728
Twenty-five	109	4.360	10.9	1.744

In general, however, the reducing-sugar values agree closely through a variation of 800 per cent. in weight of precipitate. In another experiment, 5 ml. of basic lead gave a precipitate weighing 890 mgs. per sample and a loss of 9.2 per cent. of the reducing value of the solution. In this case, with a larger sample, 2 ml. of neutral lead gave a precipitate of 887 mgs. in the same volume and no measurable loss of reducing substances. Apparently co-precipitation alone will not account for the observed losses.

RECOVERY OF REDUCING SUBSTANCES PRECIPITATED BY BASIC-LEAD CLEARING.—When it became apparent that loss of reducing substances in lead clearing is associated with the presence of lead oxide in the solution it seemed probable that an insoluble or difficultly soluble, lead oxide-reducing sugar compound was being formed. To determine, if possible, the fate of the lost reducing substances, five 200 ml. samples of a mangel-leaf extract were cleared with 10 ml. portions of basic-lead-acetate solution. After thorough washing with cold water the precipitates were washed from the filters with approximately 100 ml. of boiling water and refiltered. The combined hot-water extract was deleaded with hydrogen sulphide and reduced on a water bath to 150 cc., transferred to a volumetric flask and made to 200 ml. for a determination of reducing substances. The extracted precipitates were then combined, suspended in water and treated with hydrogen sulphide. The water-clear filtrate was made to volume and tested for reducing substances after removing the excess of hydrogen sulphide. The results are given in table V.

TABLE V

THE RECOVERY OF REDUCING SUBSTANCES PRECIPITATED BY BASIC LEAD ACETATE

SAMPLES	REDUCING SUBSTANCES IN MGS.		LOSS OR RECOVERY AS A PERCENTAGE
	Per sample	Per liter extract	
Check (low neutral)	39.2	784	0.0
High basic	33.9	678	.....
Loss	5.3	106	13.5
Hot water extraction	9.1	36.4	34.3
H_2S extraction	11.7	46.8	44.2
Recovery		83.2	78.5

With the clearing given in this experiment there was a loss of 13.5 per cent. of the reducing substances or a total of 106 mgs. for the liter used. Of this loss 34 per cent. was recovered by one extraction with hot water and

an additional 44 per cent. was recovered by treating with hydrogen sulphide. The 23 mgs. not accounted for may to a large extent be assumed to have been held by the very heavy lead-sulphide precipitate which is always washed with difficulty. The completeness of the recovery and the fact that the reducing substances were apparently unchanged strongly suggested the formation of lead oxide-reducing sugar combinations which are either less soluble than the reacting substances or which are more strongly adsorbed by various precipitates. The formation of a lead compound, particularly of levulose, has been frequently mentioned in the literature. Although our data on this point are not complete enough for final conclusions, we suggest that lead oxide is the active portion of basic lead and that both ketose and aldose sugars form lead combinations, although the former perhaps more readily.

THE RELATION OF CLEARING TO THE PURITY OF THE CUPROUS-OXIDE PRECIPITATE

When reducing substances are to be estimated from the weight of cupric copper reduced, it is commonly supposed that clearing is necessary to remove, as completely as possible, precipitable organic impurities which would otherwise be retained on the filter and weighed as cuprous oxide. Investigators who have used basic lead as a clearing reagent have justified themselves on this score. We have shown that basic-lead clearing lowers the reducing value of plant extracts or pure sugar solutions from 3 to 15 or more per cent., that the loss may vary considerably from one sample to the next, and that it is roughly proportional to the degree of clearing. The losses obtained when the clearing operation was carried out on pure sugar solutions would preclude all of the difference between neutral and basic clearing being due to a lower percentage of organic impurity in the latter case, but they do not insure the efficiency of neutral clearing. The percentages of copper in the cuprous-oxide precipitates from the low-neutral or check clearing, and the high-basic or complete clearing, are assembled in table VI. Copper was deposited on a revolving grid cathode from a mixture of nitric and sulphuric acids as previously described.

All of the low-neutral solutions showed some color and most of the apple extracts were so dark as to entirely obscure the blue of the Fehling's solution. In every case the complete clearing gave a water clear solution but the tendency was for the percentage of copper in the cuprous-oxide precipitate to be lowered rather than increased by this clearing. The question of the presence of organic impurities is not closed by these data, although we were unable to detect any traces of carbon in the solutions remaining after the copper had been removed by electrolysis. If such impurities are concerned,

TABLE VI

PURITY OF CUPROUS-OXIDE PRECIPITATE FROM EXTRACTS CLEARED WITH NEUTRAL AND BASIC LEAD ACETATE

EXTRACT	PERCENTAGE OF COPPER	
	Check clearing (low neutral)	Complete clearing (high basic)
Mangel	88.18	87.81
Apple wood no. 1	87.01	86.39
Apple wood no. 2	87.61	87.30
Apple wood no. 3	87.67	87.69
Tomato	86.82	84.52
Sweet potato	87.42	88.04
Celery	86.03	85.60
Average	87.25	86.76
Dextrose solution	88.62	88.78

however, they do not belong to the group of coloring compounds which are removed by complete clearing with basic lead acetate. The percentages of copper in the dextrose precipitates were obtained under the same conditions and indicate the error due to oxidation in drying, and loss in handling. The highest value is within 0.04 per cent. of the theoretical yield of 88.82 per cent. copper, and the lower yield is probably within the error of the procedure employed.

Three points appear significant in connection with these data. (1) The percentage of copper in the cuprous-oxide precipitate of plant extracts was lower than in the precipitate of sugar solutions—both the direction and magnitude of the difference are in accord with previous observations. (2) The copper percentage value was independent of the clearing treatment and did not vary significantly between no clearing and complete clearing, although the reducing value was considerably lowered by the latter treatments. This is contrary to the reason frequently given for the need of clearing. (3) Although the percentage of copper in the plant-extract precipitates was low, the difference was small and consistent, being of the order of 2 per cent. or less. In physiological studies a constant error of 2 per cent. will not affect the conclusions to be drawn, and the gravimetric determination of reducing sugars should be satisfactory when sufficiently large quantities of cuprous oxide can be weighed to minimize fluctuations in the weights of the crucibles used. The use of basic lead acetate, on the other hand, introduces a variable error and has no advantages when reducing substances are to be determined by their action on cupric copper.

Summary and conclusions

A procedure which has given consistent results on a variety of plant extracts is recommended for the clearing of solutions to be used in the determination of reducing substances. After removing all but the last traces of alcohol from the extract the residue is taken up in warm water, allowed to cool to room temperature and sufficient neutral-lead-acetate solution, sp. gr. 1.25, added to just form a faint, white precipitate with a drop of dilute potassium-oxalate solution. Twice this quantity is used for clearing the solution tested, to insure precipitation of reducing impurities. Without standing for more than a few minutes the cleared solution is filtered onto an excess of potassium-oxalate crystals or powder. Only cold water is used for washing the lead precipitate. This method is convenient, rapid, and gives a definite end point in reducing value.

Any heating or standing in contact with a lead solution causes a rapid destruction of reducing substances. Basic lead acetate is more destructive than neutral and gives a variable loss even in cold solutions, the magnitude of which depends upon the completeness of clearing. This makes it unreliable as a clearing reagent.

The loss of reducing substances in basic clearing appears to be due to the formation of a lead oxide-sugar combination which is less soluble than the original substances and is more readily adsorbed by the clearing or deleading precipitates. The sugars may be recovered with their reducing properties unchanged by removing the lead with hydrogen sulphide.

The completeness of clearing has not affected the purity of the cuprous-oxide precipitate. In all cases where a plant extract or a mixture of extract and sugar solution was used, the percentage of copper has been approximately 87 per cent. instead of the theoretical 88.8 per cent. No good explanation for this difference is at hand. It is pointed out, however, that the magnitude and consistency of the error makes it possible to use a correction factor for gravimetric results on plant extracts or to follow the usual calculation procedures with reasonable certainty that results so obtained will be comparable, although varying perhaps 2 per cent. from the absolute values.

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THE ADAPTATION OF CERTAIN COLORIMETRIC METHODS TO THE ESTIMATION OF NITRATES, PHOSPHATES AND POTASSIUM IN PLANT SOLUTIONS[1]

B. E. GILBERT

Introduction

The determination of the fertilizer requirements of individual crops has furnished a fertile field of investigation, and although many contributions have been made, much still remains to be accomplished before rational methods of fertilizer applications may be relied upon to produce normal crops. In the past the physiological chemist has attacked this problem chiefly from two angles. He has investigated the content and availability of the nutrient elements in the soil, and he has determined the total content of nitrogen, phosphorus and potassium occurring within the plant.

Both of these methods have failed to furnish a satisfactory basis for determining the mineral nutrient needs of plants. We cannot satisfactorily determine the quantity and kind of amendments needed on any given plot of land by making a soil analysis; neither can we tell by an analysis of the plants grown on the soil the amount and kind of fertilizer that should be added. This is no doubt due to the very large number of factors involved in crop production, especially with reference to the relationship between the plant and the soil in which it grows.

In this paper, work carried on during 1925–1926 is summarized, and a different mode of attack is suggested, which may prove helpful. Quite aside from the practical applications of this work, it is believed that other workers may find the methods here developed useful in studies of mineral nutrient metabolism.

Analytical methods

A. Preparation of plant solution

The procedure as given by Schreiner and Failyer (**9**) was adopted with certain modifications. The fresh plant tissue was shaken free from adhering soil particles, weighed and ground up thoroughly in a Nixtamel mill. The finely-ground mixture was strained through fine mesh silk and an adequate volume transferred to a small mixing bottle. With the crops investigated, a volume of 25 cc. was found to be sufficient for nitrate-nitrogen

[1] Contribution 329 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

and phosphate determinations. To this solution 1.0 gram of decolorizing carbon black for each 10 cc. of solution was added and the mixture shaken mechanically for 10 minutes. Then it was filtered by suction through an alundum extraction cone, thereby obtaining a colorless solution.

B. Qualitative methods

At first it was considered possible that chemical microscopical tests on sections of the fresh tissue might be sufficiently accurate to allow the estimation of low, medium and high contents of nitrates, phosphates and potassium. Various methods were tried as given by Chamot (3) with indifferent success. Table I shows determinations of phosphates made with ammonium molybdate reagent; table II, the estimation of potassium with sodium cobaltinitrite, and table III, the result of the use of the diphenylamine test for nitrates.

TABLE I

Microscopical observations of phosphate in tissue of field crops

Plat	Relative yield of mixture	Crop (in grass mixture)	Tissue examined	Addition of phosphorus in chemical fertilizer (lbs. per acre)	Microscopical observations (phosphate)
65	100	Timothy	Base of stem	65	Abundant
67	62	Timothy	Base of stem	0	None
69	96	Timothy	Base of stem	22	Abundant
65	100	Red clover	Stem	65	Abundant
67	62	Red clover	Stem	0	None
69	96	Red clover	Stem	22	Abundant

TABLE II

Microscopical observations of potassium in tissue of field crops

Plat	Relative yield	Crop	Tissue examined	Addition of potassium in chemical fertilizer (lbs. per acre)	Microscopical observations (potassium)
41	90	Rye	Stem	43	Abundant
43	100	Rye	Stem	21	Abundant
116	3	Timothy	Stem	0	Abundant
119	100	Timothy	Stem	124	Abundant

TABLE III

MICROSCOPICAL OBSERVATIONS OF NITRATE IN TISSUE OF FIELD CROPS

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	MICROSCOPICAL OBSERVATIONS (NITRATE-NITROGEN)
17	48	Rye	Tip of leaf	0	Trace
19	70	Rye	Tip of leaf	14	Medium
21	100	Rye	Tip of leaf	28	Abundant
23 M.G.[a]	100	Spinach	Tip of leaf	135 (16 T.M.[b])	Trace
101 M.G.	86	Spinach	Tip of leaf	84 (16 T.M.)	Trace

[a] M.G.—Market garden.
[b] T.M.—Tons straw manure.

From the nature of the microscopical methods any determination can be considered at best but an approximation of the amount of the fertilizer element in question, and while in some cases it may be possible to estimate low and high contents, differences which may exist between these two extremes cannot be determined with any assurance of accuracy. From the data it may be noted that estimates of these extremes in the cases of nitrate and phosphate were found to be feasible. It was not, however, possible to do so with potassium. No estimations as to tissue content accompanying medium fertilizer treatment seemed possible except in the case of rye with medium nitrate fertilization. With spinach, however, such was not the case. Thus the use of such methods can only be considered as qualitative in character and not of sufficient accuracy to allow the establishing of nutrient levels accompanying the production of a normal crop.

The diphenylamine reaction for nitrates seemed to allow further elaboration and so using the decolorized plant solution, diluted portions were matched on a porcelain spotting plate against solutions of known concentration of potassium nitrate. One drop of solution was used with 8 drops of reagent (0.1 gram of diphenylamine dissolved in 10 cc. of 75 per cent. fuming sulphuric acid). The color was allowed to develop for 5 minutes before reading. Approximate estimations based on this color reaction gave correlations between color developed and fertilizer treatment as shown in table IV. The diphenylamine reaction has been shown by JAEGER (8) and others to be inaccurate as a measure of nitrates due to the dependence of the color production upon an oxidation reaction. Thus the presence of other oxidation reagents than nitrate introduces an appreciable error. Realizing this difficulty the diphenylamine reaction was discarded.

TABLE IV

COMPARISON OF NITRATE-NITROGEN CONTENT OF PLANT SOLUTION AND NITROGEN ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	NITRATE-NITROGEN IN PLANT SOLUTION (PPM)
113 M.G.[a]	100	Cabbage	Midribs	75 (16 T.M.[b])	2085
112 M.G.	92	Cabbage	Midribs	50 (16 T.M.)	1737
116 M.G.	100	Tomatoes	Leaves	75 (16 T.M.)	973
115 M.G.	91	Tomatoes	Leaves	50 (16 T.M.)	814
Sec. 1 C.A.[c]	85	Corn	Stem internodes	20	Trace
Sec. 3 C.A.	100	Corn	Stem internodes	60	695

[a] M.G.—Market garden.
[b] T.M.—Tons straw manure.
[c] C.A.—Corn acre.

C. QUANTITATIVE METHODS

1. *Nitrate-nitrogen.*—The phenol di-sulphonic acid method for the estimation of nitrates as outlined by HARPER (**6**) for soils was found to be applicable when modified slightly (table V). To a volume (dependent upon the nitrate content) of the clarified solution 10 cc. of a saturated solution of silver sulphate, 0.5 cc. of normal cupric sulphate, 0.2 gram of calcium hydrate, and 0.5 gram of magnesium carbonate were added. The mixture was shaken at intervals for 5 minutes, heated to boiling, cooled, made up to volume and filtered. Of the filtrate a 10 cc.-aliquot was evaporated on the water bath to dryness, the phenol di-sulphonic acid reagent added and the remainder of the procedure carried out as outlined by HARPER. The final color was compared in a Kennicott-Sargent colorimeter against the color developed by a solution of potassium nitrate containing 1 part per million of nitrate-nitrogen.

The addition of silver sulphate is necessary due to the presence of appreciable amounts of chloride in the plant solution; copper sulphate serves to precipitate soluble organic matter; the colloidal silver chloride precipitate is coagulated by means of the calcium hydrate and magnesium carbonate, and any excess of silver ion is also thrown out of solution. This method has been found to be capable of swiftness of manipulation, ease of duplication, and an accuracy sufficient to show small differences in nitrate content.

2. *Phosphate.*—The coeruleo-molybdate method of DENIGÉS (**4**) as modified by FLORENTIN (**5**) and used for the determination of phosphate content of soil extracts by ATKINS (**2**) was found to afford a rapid and accurate

method for the estimation of the phosphate content in plant solutions (table VI). The color was developed in diluted aliquots of the clarified solution and the comparisons made by means of a Spencer-Duboscq colorimeter against a standard solution of potassium di-hydrogen phosphate having a phosphorus content of 0.25 part per million.

Since appreciable amounts of silicon occur in the plant solutions and since with certain concentrations of silicon a color similar to that obtained with phosphate is developed, it seemed advisable to ascertain to what extent silicon might interfere. Gravimetric silicon determinations were made (1) on a number of plant solutions and the silicon content found to vary from 130 to 350 parts per million of solution. The presence of silicon in solutions prepared *in vitro* from sodium silicate and containing a determined concentration of silicon, above 25 parts per million, was found to give a color which could be observed in the colorimeter. However, since dilutions ranging from 100 to 400 were found necessary in many cases to render the concentration of phosphate of sufficient dilution to allow a comparison in the colorimeter, the final concentrations of silicon in the solutions measured were always much below those liable to develop a color with the reagent. In order to further investigate the possibility of change of tint due to presence of silicon, the color was developed in solutions containing 0.25 part per million of phosphorus plus 21 parts per million of silicon, 0.25 part per million of phosphorus plus 32 parts per million of silicon, and 0.25 part per million of phosphorus, the latter having been used as a standard for comparison. The solution containing 21 parts per million of silicon gave the same depth of color as the standard while the solution with 32 parts per million of silicon was slightly deeper in color. FLORENTIN (**5**) has noted a similar differential sensitivity with phosphorus and silicon in determinations made on natural waters.

3. *Potassium.*—The method devised by HILL (**7**) and advocated by SCHREINER and FAILYER (**9**) based on the estimation of the yellow color produced by the reduction of potassium chloroplatinate with stannous chloride in the presence of free hydrochloric acid, was used (table VII). Determinations were made using 5 cc.-portions of the plant solution before clarification.

This method was found to be easily adaptable for use with the Kennicott-Sargent colorimeter and has both simplicity and accuracy in its favor. However, certain precautions were found necessary. The potassium content of the solution under investigation must govern the volume used, as too great a concentration of chloroplatinate will interfere with the final color produced on reduction. Extreme dilution of the chloroplatinate solution before reduction causes a turbidity to develop which interferes with the final color determination.

It should be noted that extreme accuracy is not claimed for these methods due to all the errors accompanying colorimetric methods of which the occurrence of "off-tints" is perhaps the most difficult to control. But with careful manipulation and the observance of certain precautions such as the development of the standard and sample colors at the same time, these inherent errors may be minimized. On the other hand, however, it is claimed that the use of these methods enables the worker to estimate small concentrations, establish small differences, and give him a means of securing ease and rapidity of manipulation so that many samples may be determined with a minimum of time and labor.

Analytical determinations

Crop plants growing under similar conditions as to climate, soil P_H, and period of growth were chosen for analysis. In so far as was possible crops were chosen in which the only variable factor during growth was the amount of one fertilizer ingredient. Each sample consisted of as many individuals as was consistent with the material obtainable, and ease of manipulation. During the months of November to February inclusive, plants were also grown in the greenhouse. Soil was transferred from three field plats to the greenhouse. The soil from one plat was known to be low in active P_2O_5; that from the second low in nitrate-nitrogen; and the third low in K_2O. Determinations for active P_2O_5 and nitrate-nitrogen were made on the first two soils. The soil low in phosphate yielded 44 parts per million of active P_2O_5 after a 1-hour extraction with 2N acetic acid. The low nitrate soil was found to contain 11 parts per million of nitrate-nitrogen. To each of

TABLE V

COMPARISON OF NITRATE-NITROGEN CONTENT OF PLANT SOLUTION AND NITRATE-NITROGEN ADDED AS CHEMICAL FERTILIZER

PLAT	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	NITRATE-NITROGEN CONTENT IN PLANT SOLUTION (PPM)
Greenhouse	Lettuce	Leafy	0	Trace
Greenhouse	Lettuce	Leafy	60	225
Greenhouse	Lettuce	Leafy	120	523
Greenhouse	Tomatoes	Leaves and petioles	70	560
Greenhouse	Tomatoes	Leaves and petioles	53	350
Greenhouse	Rye	Leafy	0	33
Greenhouse	Rye	Leafy	60	50
Greenhouse	Rye	Leafy	120	80

these soils three levels of each fertilizer element were added and crop plants grown. The quantitative methods as outlined above were employed and a few of the results obtained are given in tables V to VII.

TABLE VI

COMPARISON OF PHOSPHORUS CONTENT OF PLANT SOLUTIONS AND PHOSPHORUS ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF PHOSPHORUS IN CHEMICAL FERTILIZER (LBS. PER ACRE)	PHOSPHATE-PHOSPHORUS CONTENT IN PLANT SOLUTION (PPM)
108	82	Corn	Stem tips	0 (2.3 T. M.[a])	2
107	90	Corn	Stem tips	26 (2.3 T. M.)	34
112	100	Corn	Stem tips	52 (2.3 T. M.)	78
79	90	Corn	Stem tips	39 (1924)	43
77	100	Corn	Stem tips	59 (1924)	60
Greenhouse		Turnips	Leafy	0	32
Greenhouse		Turnips	Leafy	60	42
Greenhouse		Turnips	Leafy	120	93

[a] T. M.—Tons shavings manure.

TABLE VII

COMPARISON OF POTASSIUM CONTENT OF PLANT SOLUTION AND POTASSIUM ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF POTASSIUM IN CHEMICAL FERTILIZER (LBS. PER ACRE)	POTASSIUM CONTENT IN PLANT SOLUTION (PPM)
119 M. G.[a]	100	Celery	Stems	83 (16 T. M.[b])	900
89 M. G.	89	Celery	Stems	124 (16 T. M.)	1100
113 M. G.	84	Beets	Roots	83 (16 T. M.)	612
83 M. G.	100	Beets	Roots	124 (16 T. M.)	812
115 M. G.	93	Spinach	Leaves	83 (16 T. M.)	1350
86 M. G.	100	Spinach	Leaves	124 (16 T. M.)	1450
Greenhouse		Lettuce	Leaves	0	1641
Greenhouse		Lettuce	Leaves	100	2116
Greenhouse		Lettuce	Leaves	199	2777

[a] M.G.—Market garden.

[b] T. M.—Tons straw manure.

Discussion

The individual colorimetric methods have already been considered. There remains the discussion of the practical application of these methods. It is recognized that any method of determining the fertilizer needs of plants is beset with difficulty. The data at present are insufficient to give us any idea of the amount of variability to be expected in the concentration of mineral nutrients in the sap of normal crop plants. With reference to mineral nutrient cultures, it has been shown that there is no single best mineral nutrient ratio for the growth of any kind of plant in such culture solutions. Over rather wide ranges of variations of these ratios the growth of plants is about equally good. Similarly we do not know at present how wide may be the variation of the ratios of N:P:K in the cell sap, and still have normal crop production. However, it was felt that determination of the soluble nutrients in the cell sap might furnish a more reliable clue to the nutrient needs of plants for normal growth than the older methods did. There are some indications in the data presented that the methods here employed may have value in this connection. Thus in tables VI and VII there is some correlation shown between yield and the amount of nutrient element found in solution in the cell sap. Hitherto attempts have been made to secure information regarding nutrient levels mainly by making total analytical determinations on plant tissues. One would expect that a determination of the amount of nutrient elements actually present in solution, and capable of entering into metabolic combinations, should be a better method of delimiting such nutrient levels. This should be true, particularly if determinations are made at intervals throughout the growth of the plant, and coupled with growth measurements and total yield. If by such methods we can determine what constitutes a proper nutrient level for normal crop production, and how to change the nutrient level by soil amendments, these same methods may be of use in maintaining these levels by providing information as to the amount of fertilizer needed, and the proper time for application during the development of the plant.

Summary

In this paper are discussed certain colorimetric methods for the determination of nitrate-nitrogen, phosphate-phosphorus and potassium in the plant solution and the practical applications arising from their use.

1. *Qualitative.* The usual chemical microscopical tests were found to be incapable of use in the establishing of low, medium and high nutrient levels in crop plants.

2. *Quantitative.* The following methods were found to be applicable to determinations with plant solutions:

(a) Nitrate-nitrogen.—Phenol di-sulphonic acid method.
(b) Phosphate-phosphorus.—Coeruleo-molybdate method.
(c) Potassium.—Reduced chloroplatinate method.

3. The application of these methods to the establishing of optimum nutrient levels of the three fertilizer elements within the plant as indicative of a normal crop is discussed.

The writer is indebted to Dr. B. L. HARTWELL for his kindly criticism and encouragement during the progress of this work.

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KINGSTON, R. I.

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BRIEF PAPERS

SOME FURTHER NOTES ON THE HYDROLYSIS OF STARCH GRAINS UNDER POLARIZED LIGHT

(WITH ONE PLATE)

The interest taken by workers in America in the effects of radiation of all kinds and in particular of polarized light, together with the confirmation of my results, kindly expressed by Prof. MACHT and Mr. MORRISON, lead me to hope that some fuller account of these experiments may be acceptable.

The first paper published in Dec., 1924,[1] was necessarily of a preliminary character, but recent work has completely confirmed these early results and has made the whole process much clearer.

Like all new ideas, this work has not escaped criticism,[2] to which a reply[3] has been given, and it is hoped that the following added notes and photographs will show that the views put forward by our critics are quite untenable.

1. As stated in the above mentioned paper, washed and centrifuged starch grains were suspended in water, to which weak diastase was added and a few drops of the starch suspension were mounted on a microscope slide placed over a Nicol prism. Two controls were always arranged, one in ordinary light, and the other in darkness. It has been suggested that the whole effect was due to pressure of the cover slip, but this factor would have affected the controls also.

2. In the second place, it is most important to note that the whole process was watched carefully under the microscope for four or five hours, the changes were noted and *drawings* were made. Unless this is done, a superficial observer may easily miss the results, particularly if no cover slip is used, as the grains on hydrolysis become lighter, exude their contents, and the empty coats float to the edge of the slide or vessel.

3. The experiment was repeated nearly twenty times, and the result was shown to nine or ten different observers, chiefly professors and lecturers of the University of London.

[1] BALY, E. C. C., and SEMMENS, ELIZABETH S. The selective photochemical action of polarized light.—I. The hydrolysis of starch. Proc. Roy. Soc. London B. **97**: 250–253. 1924.

[2] JONES, NEILSON. Polarized light and starch grains. Ann. Botany **39**: 651–653. 1925.

[3] BALY, E. C. C., and SEMMENS, ELIZABETH S. Selective action of polarized light upon starch grains. Nature **116**: 817. 1925.

4. The first change was seen as a light transparent streak, evidently *within* the grain and starting from the hilum. This increased, and branches were formed extending to the exterior. These are seen on plate VIII, figs. 5 and 6.

5. The whole grain became lighter and more transparent and the edges began to split. Figs. 7 and 8. (This is also seen in fig. 3 where the effect was produced by polarized light alone, acting on well-washed grains in distilled water.)

6. The grain started to swing very slowly from side to side and in some cases moved its position, owing probably to exudation of the hydrolyzed contents of the grain and to changing surface tension. This fact shows that the change observed could not have been due to pressure of the cover slip.

7. Under crossed Nicols the humineseence of the grain gradually disappeared from the center towards the periphery. Fig. 9.

8. The familiar black cross became blurred in the same way and finally disappeared.

9. On drying, a cluster of crystals formed *at the point where the grain had been* and only the empty shrivelled or broken coat or shell remained. This sometimes became so transparent as to be nearly invisible. Figs. 10 to 12. The arrangement of these crystals most probably depends on the shape of the original starch grain.

10. On addition of Fehling's solution, as before stated, the presence of a reducing substance was indicated. The photomicrographs, figs. 1 and 2, are submitted as showing the results better than those in the original paper. They were not published at the time, as one of the controls was spoiled.

In both controls, however, the whole field on the slide was searched and only two grains were found imperfect on each slide. In fig. 1, over the polarized light, the grains were commencing to break all over the field exposed.

Fig. 3 illustrates the change within the grain before any serration has taken place, except that little canals are forming to the exterior. This result was obtained with long weak exposure to polarized light. No diastase was added to the starch suspension. In fig. 13 this change has gone further; the contents of the grain are forming crystals and the shell is beginning to crack. In fig. 4 and fig. 14 the coat has broken down and the hydrolyzed contents have exuded into the surrounding liquid.

In conclusion, it may be noted that if, as Professor JONES suggests, all these results are due to accidental pressure of the coverslip (even when the exposure took place in a flask!) then there is a by fortune to be made in the sugar industry. The only requisites are a few tons of corn or potatoes and a steam-roller!—ELIZABETH SIDNEY SEMMENS, *Bedford College, University of London.*

EXPLANATION OF FIGURES.

FIG. 1. Potato starch exposed to light polarized by a Nicol prism. All stages of hydrolysis are present. (See figs. 5–12.)

FIG. 2. Control of potato starch exposed to ordinary light. Grains A and A′ appear to be fractures, as there is no transparency in the middle, which is the mark of hydrolysis.

FIG. 3. Starch grains exposed in flask to polarized light through base of flask, showing hydrolysis of contents, probably to dextrin. C, C: canals to exterior.

FIG. 4. Starch grains exposed to polarized light, with diastase, showing exudation from grain. The coat of the grain is left in the center.

FIGS. 5–12. Typical stages of hydrolysis of starch grains exposed to polarized light, with diastase. Note transparent streak in fig. 6, the rod-like crystals from the shell of the grain in fig. 10, and the radiating crystals surrounding the shell in figs. 11 and 12.

FIG. 13. Washed starch grain in distilled water, after several days' exposure, in a flask, to polarized light. Note crystals formed inside; also slits in the coat.

FIG. 14. Starch grains, prepared and exposed as in fig. 1. Note exudation from the grain crystallizing out.

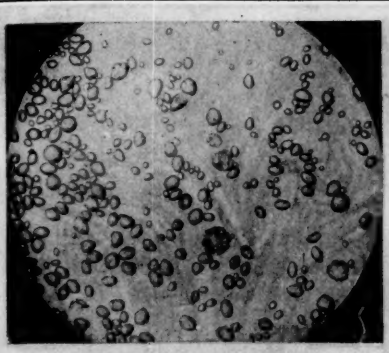


Fig. 1

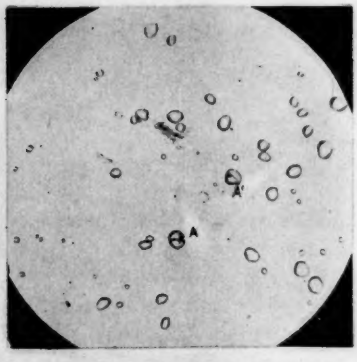


Fig. 2

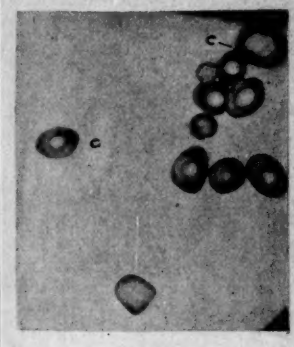
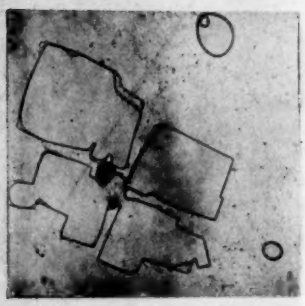


Fig. 3

Fig. 4



Figures 5 to 12

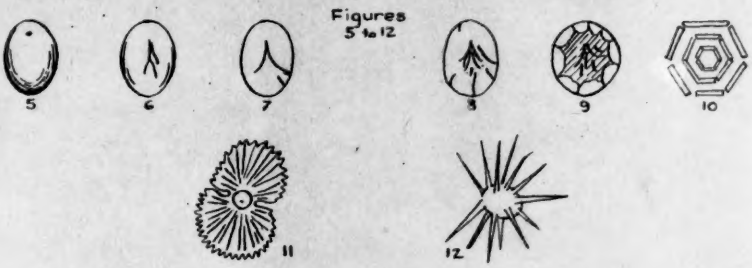


Fig. 13

Fig. 14

SEMMENS—HYDROLYSIS

A CONDUCTIVITY CELL FOR CONTINUOUS MEASUREMENTS OF RESPIRATORY RATE

(WITH ONE FIGURE)

The conductivity method is very desirable for continuous readings of the rate of CO_2 production when rapid fluctuations in the rate of respiration are under investigation. There have been devised by CAIN and MAXWELL[1] and by SPOEHR and MCGEE[2] conductivity cells which make use of the change in conductivity of $Ba(OH)_2$ solutions for the estimation of carbon dioxide. The apparatus here described (fig. 1) has some advantages

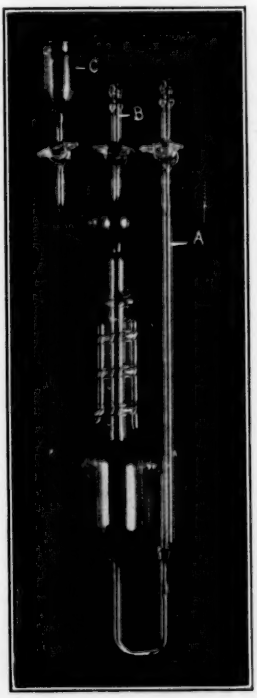


FIG. 1. Conductivity cell for respiration studies. See text for description.

[1] CAIN, J. R., and MAXWELL, L. C. An electrolytic resistance method for determining carbon in steel. Jour. Ind. Eng. Chem. **11**: 852–860. 1919.

[2] SPOEHR, H. A., and MCGEE, J. M. Studies in plant respiration and photosynthesis. Carnegie Inst. Washington. Publ. no. 325. 1923.

over the former types since it can be made small and compact and is not easily breakable. The apparatus can be shunted into a closed system of air circulation or the CO_2 may be drawn through it by vacuum.

The gas enters by the long tube (A) at the right, rises through the spiral in interrupted bubbles mixed with the $Ba(OH)_2$ solution, and passes out the center tube (B) which is provided with a bulb to break up the films. The absorbing solution is thoroughly mixed and continually circulated through the spiral. The funnel side tube (C) is useful for filling the apparatus. Connections to the electrodes are made through glass tubes at the sides. The cell is made of pyrex glass and the change of the capacity factor is found to be negligible since each electrode is protected from the deposition of $BaCO_3$ on it by a projecting glass collar. The electrode vessel is filled with a known volume of $Ba(OH)_2$ solution (100 cc.) so that the level stands below the top of the spiral when the cell is in operation. The concentration of the $Ba(OH)_2$ solution may be varied within the limits of the capacity of the electrodes. Usually N/20 to N/200 $Ba(OH)_2$ is used. The cell is arranged for immersion in a water thermostat.

We have found this cell very useful for measuring quick changes in the rate of respiration of tissues resulting from treatment with ethylene and other gases.—R. B. Harvey and L. O. Regeimbal, *University of Minnesota.*

CITATION OF LITERATURE

As science progresses there is a constantly increasing number of investigators who need the services of scientific periodicals in placing the results of their research before the public. This is true of botany and plant physiology, just as it is of all the other branches of science. The volume of literature produced by this constantly augmenting army of workers has grown correspondingly. One is reminded, in this connection, of those autocatalytic processes in which a product of the reaction catalyzes, and increases the rate of the reaction. We have the machinery for investigation in operation, and one of the products of this system is the trained investigator. He is the catalyst, who is constantly accelerating the rate and volume of scientific output.

With this increase in the number of workers and volume of literature, the editors and publishers of journals find themselves confronted by various serious problems. One of these is the citation of scientific literature. In the early years it was necessary to bring together far scattered materials, and focus attention upon the bearings of these fragmentary contributions. So the custom grew up of citing the literature rather completely. It was a useful and necessary part of scientific publication. Then the literature was scanty in all fields; today there are only a few fields in which the published

literature is scanty, especially in connection with the larger problems of biology. It must be perfectly obvious that we shall not be able to continue indefinitely the custom of giving complete citations to the literature of any given field. We shall soon reach the place where the literature citations will occupy more space than our contributions warrant. This situation should be given careful and deliberate consideration by investigators, editors and publishers.

Investigators can render assistance by using more discrimination in the citation of papers. There is no particular virtue in the long list of citations as such. Occasionally one sees the results of over-zealous listing of literature which hardly deserves mention, and the inclusion of papers that have but little bearing on the problem under discussion. The writer has in mind a case which came to his attention some time ago. It is mentioned only to provide a concrete example of a method to be avoided. Buscalioni and Pollacci in 1904 published a lengthy paper on "The anthocyanins and their biological significance in plants." The references to the literature numbered well toward 900 even at this early date, and occupied about 30 pages, just for citations alone. Among these citations was noted one on "Blue Ridge Blossoms." While some of the plants mentioned in this species list had anthocyanin-containing flowers, the paper was probably included because it had the word *blue* in the title. The paper cited had nothing to do with the biology of the anthocyanins. A discriminating consideration of the value of papers would have led to the omission of those which had no bearing on the problem.

It is necessary, of course, to *know* the literature as completely as possible. Nothing can quite take the place of a complete bibliography in the hands of the individual workers. But when we come to report our results we can conserve space by limiting ourselves strictly to the citation of only such papers as cannot be omitted from the discussion without loss in value.

It is frequently possible to reduce the number of citations by reference to other recent papers where most of the earlier papers have been summarized. By such measures, and judicious choice of citations, one may greatly reduce the number of papers that must be mentioned, and so reduce the cost of publication.

The editors and publishers of this journal invite the cooperation of those who use its pages as a medium of communication with other botanists in a program of true economy, which does not sacrifice quality, but which eliminates as far as possible any unnecessary waste of labor and space.—C. A. SHULL, *Editor-in-Chief*.

NOTES

The International Congress of Plant Sciences.—It is now possible to mention the names of some of the distinguished foreign scientists who will attend the Ithaca Congress, at Cornell University, Aug. 16–23, 1926. Plant physiologists will note with pleasure the inclusion of such names as Stoklasa, Lepeschkin, Maximow, Chodat, Němec, Winogradsky, Prianishnikow, Lubimenko, and many others. There are still others who would come if they could secure financial aid through giving lectures before American scientific audiences. It would be a very appropriate and gracious form of hospitality to extend invitations to some of these noted men, who are hesitating to come because of financial reasons, to lecture before the students and faculties of our respective institutions. Can we not use this opportunity to foster a warmer international cordiality among scientists? Science knows no national boundaries nor racial prejudices. It serves humanity impartially. We want to give our distinguished guests a real heart-warming experience during this visit to America.

Those who would like to extend invitations to visitors who would thus be enabled to attend the Congress can secure information as to where such assistance would be welcome from Dr. B. M. Duggar, Chairman of the Executive Committee, and General Secretary of the Congress, who should be addressed, National Research Council, B and 21st Sts., N.W., Washington, D. C.

Membership in the A. A. A. S.—The Council of the American Association for the Advancement of Science voted at the Kansas City meeting to remit the entrance fees to all members of affiliated societies during the next three years. This is an opportunity which will no doubt appeal to the new members of all of the affiliated societies. Election to membership in one of the affiliated societies should be considered an invitation to membership in the general Association.

Life Membership Fund.—"The American Society of Plant Physiologists . . . has established a life membership fund of $2,000, in honor of Dr. CHARLES REID BARNES, who was, at the time of his death in 1910, Professor of Plant Physiology at the University of Chicago. The fund has been established in accordance with plans accepted by the Society at its second annual meeting at Kansas City. The interest of this fund will be used each year to elect to life membership in the Society some member whose contributions to Plant Physiology make him most worthy to receive the honor.

The members so elected will be known as the Charles Reid Barnes Life Members. The first election will be held in connection with the third annual meeting at Philadelphia in December, 1926.''—Science: Feb. 26, 1926.

Summer Meeting.—The Corn Belt Section of the American Society of Agronomists will meet at the University of Minnesota, July 15–17, 1926. Last year the American Society of Plant Physiologists met with the Agronomists at Michigan State College, East Lansing, and the joint meeting was greatly enjoyed by those in attendance. An invitation has been extended to the Physiologists to meet again with the Agronomists. A little later the Horticulturists are planning a meeting at the University of Minnesota. It would have been a splendid arrangement if these several groups could have met together, since the fields are so closely allied, but such a joint meeting of all three probably cannot be held this year.

In view of the fact that the International Congress of Plant Sciences meets at Ithaca in August, it is hoped that no one will sacrifice the meeting at Ithaca for other meetings. However, these regional meetings of allied groups for good fellowship and mutual stimulation are worth while, and it would be fitting for the Physiologists to hold a joint regional meeting with the Agronomists at Minneapolis. Announcements will no doubt be sent out to the members if such a regional meeting is arranged.

The Purdue University Section.—To Purdue University goes the honor of organizing the first Local Section of the American Society of Plant Physiologists. The organization of such a section requires the presence of ten or more members in the local group, and Purdue is to be congratulated on having so large a group interested in the promotion of research in plant physiology. The Purdue Section has been meeting weekly, the meetings being delightfully informal, and the group is enthusiastic over the results of the early meetings. Dr. E. B. MAINS is the local Vice President, and becomes *ex-officio* a member of the Executive Committee of the Society. No other officers have been elected. It is a great pleasure to welcome this first Local Section, and we hope that many such sections may be organized among the investigators in our larger universities.

The University of Minnesota Section.—An application for a Local Section at the University of Minnesota has been received. This application will be acted upon in due time. It is very encouraging to see the plant physiologists uniting to encourage one another in the investigation of important physiological problems, and to enjoy the fellowship of common interests. There are many ways in which such a group of investigators can cooperate to the advantage of each individual in it, and of the program of research as a whole. It should be definitely easier to maintain the spirit of

enthusiastic research where men are banded together in this way for mutual helpfulness.

Research Libraries.—Every investigator finds it necessary to own a library made up of books which are indispensable aids to research. Among these there are always some which must be imported. Those who need the services of an importer will find G. Schmidt, 415 Woodland Ave., Leonia, N. J., and B. Westermann Co., 13 West 46th St., New York, courteous and prompt in caring for import orders. Those who desire to send directly to Europe will find Oswald Weigel, Königsstrasse 1, Leipzig, Germany, reliable and accommodating to purchasers of books.

Chemical Bibliographies.—The National Research Council, Washington, D. C., has published a bibliography of bibliographies on chemistry and chemical technology. The work has been compiled by WEST and BEROLZEIMER, and is published as Bulletin no. 50 (308 pp., $2.50). There are a number of sections, including general bibliographies, abstract journals and year books, general indexes of serials, bibliographies of special subjects, and personal bibliographies.

The research chemists and chemical physiologists will find this work of assistance in locating bibliographies relating to any subject in which they are interested. The literature lists from research papers, and even footnote bibliographies have been compiled, giving the name of the author, title of paper, place of publication, and frequently the number of citations listed.

The references are classified according to proper subject headings alphabetically arranged. There are about 2,400 subject headings, 7,500 author entries, and approximately 10,000 individual bibliographies. The volume should find a place in the library of every one who may need to consult the literature of chemistry. Omissions no doubt occur, but the book offers an excellent starting point for the necessary delving into the literature in connection with research.

Chemical Plant Physiology.—The rapid advances made in plant physiology during the last 25 years constitute a revolution almost as extensive as that which occurred in chemistry during the last quarter of the eighteenth century, and as striking as the revolution which has taken place in physics during the last three decades. In the first volume of his new Lehrbuch der Pflanzenphysiologie, KOSTYTSCHEW points out the fact that a comparison of his book with the same chapters in PFEFFER shows that little is left of the physiology of 20 years ago, "ein kaum zu erkennendes Gerüst," and that innumerable new problems have come up for solution. These enormous changes have not yet been recorded sufficiently in text books, especially in

English. KOSTYTSCHEW has attempted, in this volume on chemical physiology, to present the new science in appropriate form. The eight chapters in volume I are logically arranged, and deal with the following topics: The foundations of chemical plant physiology; the assimilation of sunlight energy by green plants, and the primary synthesis of organic matter; chemosynthesis and the assimilation of molecular nitrogen; the nutrition of plants by organic compounds; the nutrition of plants by ash elements and its significance; carbohydrates and proteins, their transformations in the plant; the secondary plant constituents; and respiration and fermentation.

The book is thoroughly modern in its point of view, and should be examined by every physiologist. KOSTYTSCHEW still clings to the ten necessary elements, a point of view that is becoming untenable as we more carefully exclude elements needed in minute quantities. MCHARGUE'S work on manganese is not mentioned in the discussion of this element, and evidence is accumulating that other elements are necessary in minute amounts, possibly as small as a part in several millions of water. Perhaps we should no longer try to list a definite number of required elements.

Official and Tentative Methods of Analysis.—The 1925 edition of this well known publication of the Association of Official Agricultural Chemists is now available for distribution. The revision has been thorough, and includes the additions, deletions, and other changes made at the meetings of the Association from 1919 to 1923. The book contains nearly 100 pages more material than the 1920 edition, and two additional chapters, on agricultural liming materials, and gelatin. The arrangement of chapters has been changed at several places, soils now following immediately after fertilizers, after which comes the new chapter on liming materials. This is a better arrangement. The price is $5.00, and may be ordered from W. W. SKINNER, Secretary, Box 290, Pennsylvania Avenue Station, Washington, D. C.

Chemical Action of Ultraviolet Rays.—A useful summary of the enormous literature on ultraviolet radiation has been compiled by ELLIS and WELLS. Plant physiologists will find many of the chapters illuminating, although they deal with other than strictly biological effects. The book contains fifteen chapters, the most valuable of which are those dealing with photochemical mechanism, photochemical and photolytic reactions, photosynthesis, sterilization, and the biologic effects. A few errors will be found by the critical reader, and one has the impression that the authors have taken too seriously some of the highly theoretical and hypothetical papers on the relation of ultraviolet radiations to photochemical reactions occurring

in plants. Any student of the ultraviolet as a factor in biological processes will find it an indispensable aid, in spite of any shortcomings it possesses. It is published by the Chemical Catalog Co., New York.

Physiological Basis of Drought-Resistance of Plants.—This volume by Prof. N. A. MAXIMOW, published in Russian by the Institute of Applied Botany and New Cultures, Leningrad, 1926, represents a critical survey of the literature on water balance and drought resistance, supplemented by investigations of the author and his coworkers in this field. The first part deals with the processes of absorption of water in the soil; the second, with transpiration phenomena. The third part discusses water balance, water deficit, and the problems of drought resistance. The book is valuable in emphasizing the anatomo-morphological characters as well as the physiological characters of drought-resistant crops. While the main text will remain a sealed book to most American students, it has a brief abstract in English, fifteen pages, at the close. Negotiations are under way for an English translation, slightly abridged, under the editorship of Prof. Yapp, of the University of Birmingham. The Russian edition may be obtained by addressing the Institute, Rue Herzen 44, Leningrad, U. S. S. R. The price is $2.50.

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